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THE DEVELOPMENT OF THE REPRODUCTIVE DUCTS AND CANALS IN THE FREE-MARTIN WITH COMPARISON OF THE NORMAL.

A DISSERTATION

Submitted to the Faculty
of the Ogden Graduate School of Science
in Candidacy for the Degree of
Doctor of Philosophy.

DEPARTMENT OF ZOOLOGY

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By

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The Development of the Reproductive Ducts and Canals in the Free-martin with Comparison of the Normal.

INTRODUCTION

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DEVELOPMENT OF ORGANS AND SYSTEMS

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SUMMARY AND DISCUSSION.

INTRODUCTION.

In cattle a female born co-twin to a male is sterile in the majority of cases and is known among cattlemen as a "free-martin". By some authors all females of two-sexed twins in cattle are called free-martins. The proportion of the cases in w ich such female is normal is given as follows; Numan ('44) one in eight, Lhör ('13) six in ninety-one, Lillie ('23) six in thirty-nine. These with one in thirty-six as found by the author, make a total of 20 in 287, or about 1 in 14. The reason for the sterility is quite conclusively shown by Lillie ('16,'17,'23) to be due to definite and sufficient vascular anastomosis between the circulations of the twins in the placental membranes, in the absence of which the female is normal. The male is always normal.

By the older authors the free-martin was classed as an hermaphrodite, or pseudo-hermaphrodite, as modified male (Numan, '44)(D. Berry Hart, '10)(Bateson, '13), or as modified female (Numan, '44). The last named author classed them as males or as females, according as the organs are more or less female or male in type. Spiegelberg ('61) classed them as transverse hermaphrodites.

Numan ('44) describes the case of a supposed free-martin which he obtained from a dealer when a year old, which had an apparently female set of external genitalia, moved ventrally along the body-wall, with an accessory urethral opening dorsal to it. The internal urogenital organs were decidedly male, but no evidence can be got from his figure as to the development of

an udder or scrotum, and the latter would be a strong criterion of maleness. Keller and Tandler ('16) state incidentally that they have noted modifications of the external genitalia in the male direction, but they do not describe them. A case is described and interpreted as abnormal male by Magnusson ('18) of a foetus twin to a normal bull calf, which had no prepuce, scrotum, vulva, vagina, developed cervix, fallopian tubes or hydratid, and no prostates, but which had a short hidden penis in the urethra, an udder and teats, a uterus with horns and body, seminal vesicles, vas deferens, epididymis, and partially descended gonad. With these few possible exceptions, the female is modified in a remarkably definite and limited manner, so that her internal genital organs are made over in the male direction, and her external ones remain distinctively female.

As to the cause of this remarkable situation, Lillie ('16,'17), in America, and Keller and Tandler ('16) in Europe, quite independently, arrived at the same conclusion that the free-martin condition is due to the action of a male hormone, from the male co-twin, passing by vascular anastomosis of the placental vessels to the female, which causes modification ofthe internal generative organs of such female in the male direction, without in any way interfering with the normal development of the male co-twin. Keller and Tandler do not say where they think the hormone is produced nor when it begins to act. Lillie ('17) postulates that since the interstitial cells of the mammal testis develop long before those of the ovary, they are probably the seat of male hormone production. Lillie and Bascom ('22) and Bascom ('23) show that the interstitial cells of normal male cattle embryos appear between 2.7 and 3.0 cm. stages, which is about the time of the initiation of external sexual

differentiation, while they do not appear in females till 37.0 cm., which is near the time of birth. Lillie ('23) shows that the hormone is already effective in a free-martin of 2.75 cm., and points out that zygotic sex-determining factors are also in a certain degree sex-differentiating factors in mammals as in insects, and that, while in the female these factors are probably not reinforced by sex-hormone action till about the time of birth at earliest, in the male they are reinforced early. He seems to think that the hormone may be introduced in the case of the free-martin before sexual differentiation, but this can hardly be true for the appearance of interstitial cells is in itself a sexual differentiation, and in the free-martin, while the anastomosis does take place as early as the 1.8 cm. stage, Lillie ('23), and probably as early as 1.0 cm., confirmed in the writer's collection, the hormone cannot be introduced before the appearance of the cells that produce it. In the free-martin while the effects may appear early in some cases and are the same effects as produced in the male, in other cases, they do not appear till the female has undergone a considerable amount of sexual differentiation. Presumably the hormone has not been introduced till near that stage.

To date no exact and minute anatomical comparisons of the earlier stages of normal male and female embryonic and foetal development have been made with reference to the differentiation of the sex-ducts and urogenital canals. This, with the same sort of study of the free-martin, is necessary, of course, as a basis for the complete analysis of the causes of the free-martin condition and its variation.

Bergschicker ('1[illegible]) worked out the development of the female Wolffian and Müllerian ducts and genital apparatus in cattle and describes the changes found in the normal female. Lillie ('17, '23) took up the gross anatomical modifications of the free-martin as compared with some males and females as a basis for his theory of the free-martin, and a minute study of a 3.75 cm. free-martin gonad and external genitalia. Chapin ('17) includes in her paper on the foetal free-martin gonad some notes on the ducts adjacent to the gonad. All earlier studies consist for the most part of anatomical descriptions of individual free-martins without any attempt to correlate the situation with normal modes of development.

Gross dissection cannot show the real state of the ducts and their derivatives and definite knowledge of these conditions at successive stages in both normals and free-martins, and a comparison of one with another at equivalent stages, is necessary as the basis for decisive conclusions as to the course and causes of this modification.

At the suggestion of Professor F. R. Lillie, the writer started to investigate the normal development of the sex-ducts and genital canals in male and female calf-embryos, up to a stage when the organs are well started toward their definitive condition, for the purpose of comparing the modes of development in the two sexes. As the collection of material for the study outlined above went on, free-martins were taken which fitted into the series, and a comparison of five of them with the normals has been incorporated into the study. In one case, the comparison of two free-martins of the same size has been made to determine and correlate the variations found in those of the same size.

The writer wishes to express his gratitude to Professor Lillie for his suggestion of the original problem and for unfailing and kindly advice and instruction throughout the work.

Starting with the premises as a working hypothesis, that the free-martin was a zygotically determined female first, and that she would have had the normal female mode of development throughout, but for the action of the male hormone injected into her in utero by way of the placental vascular anastomosis, the investigation has been an attempt to find out the effect of this male hormone on the female embryonic development. This is done by attempting to analyse the series of natural experiments presented, with reference to the factors influencing the varying results, and different degrees of the same result, produced, using the normals as controls. An attempt has therefore been made to test the working hypothesis and to answer the following questions in addition;-

(1) What are the normal female and male modes of development?

(2) What changes from the expected normal female mode have resulted?

(3) What factors underlie the variations in the observed effects of the hormone in the free-martins studied?

(4) Is the action of the hormone limited to any sets of structures? If so, to what degree, and in what manner, does it affect each set of organs?

This investigation leads to the following general conclusions in addition to producing evidence that the working hypothesis was sound;-

(1) The normal female development of the Müllerian duct-derivatives, of the distinctive secondary sex- cords in the cortex of the ovary, and late development of secondary tunica albuginea; the development of the female type of sinus urogenitalis, with suburethral diverticulum, vestibule, vulva, bulbo-vestibular glands, and clitoris; and the development of a round ligament; the de-

generation of the Wolffian duct derivatives; and the early loss of connection of the cranial group of mesonephric tubules to the Wolffian duct, would have gone on apparently under the control of zygotic factors, unaided by reinforcement by sex-hormone action, at least till near the time of birth.

The normal male mode consists of the development of the Wolffian duct derivatives, the early separation of the sex-cords from the epithelium of the gonad, and failure of secondary sex-cords to form; the early degeneration of the Müllerian duct derivatives; the development of the male type of urogenital sinus derivatives, without suburethral diverticulum, and with short urethra proper, and long prostatic and cavernous urethrae, sigmoid flexure, bulbo-urethral glands, and distinctive little pouch at the bend of the urethra; the ventral migration of the penis and growth of the perineum; the development of scrotum and distinctive gubernaculum and saccus vaginalis; under the control of zygotic factors reinforced early by male hormone action.

(2) The changes from the expected female mode are; failure to appear, or degeneration, of the secondary sex-cords in the gonad; degeneration of the Müllerian duct derivatives as in the male, but showing signs of having developed as in females to varying degrees before degenerating; the development of the Wolffian duct derivatives as in males, with signs of early partial degeneration as in females; practically no change in the female mode of development of the urogenital sinus derivatives; and development of a more or less male type of gubernaculum and saccus vaginalis. All show the effects of the male hormone parallel to those in the male.

(3) From the results brought out by this study it is believed thatthe factors underlying the variations in degree of effect of

this hormone in different free-martins of the same size and in those of different sizes are;- (a) Time of initiation of hormone action, which in turn depends on time of hormone production in the male co-twin, or on time of completion of placental vascular anastomosis. (b) The length of time the hormone has been acting, just as in the normal male, where time is a factor in the state of the ducts, etc., at any stage, but with a variable condition of the organs upon which it has to begin action.

(4) In all cases observed, the action of this hormone on the organs of reproduction is limited to organs of mesodermal origin, and fails to affect those of ectodermal origin, except, perhaps, to cause slightly earlier appearance of para-urethral ducts, which, however, are not male in type, but definitely female. External genitalia, in all cases observed, are unaffected in the male direction. Though it is not lost sight of, that some such modifications are described by other observers, it is believed that they may well be of zygotic origin and not due to male hormone action in uterine life. Such modifications appear in single-born individuals, and are therefore to be expected in about the same proportions among two-sexed twins, whether with hormone action or without.

The apparent specificity in the hormone action is due to specific reactions of the tissues affected, in that some are stimulated to increased development and differentiation by it, while others are inhibited and caused to degenerate, and still others appear unaffected. The changes in general body shape noted in free-martins, and in equivalent organs in the two sexes, in which the free-martins resemble males somewhat, would show that other parts beside sex-organs are affected differentially. If there are parts unaffected, they may well be zygotically controlled or past a critical

stage before the hormone reaches them. This would also account for the fact that complete sex-inversion is not found in free-martins. The fact that the affected organs in free-martins lie anterior to the vestibule, will account for their being classified by early writers as transverse hermaphrodites.

This study also shows that, not only does the male hormone inhibit the development of Müllerian duct derivatives and ovarian secondary sex-cords, but it causes active degeneration of those structures when they are already formed in the female embryo. This would lend support to the idea of Steinach ('16 and later) and Lillie ('17) that the testis, through its hormone, exerts an action that is antagonistic to the ovary, at least in early embryonic life, and would show that Sand ('19) and Moore ('21) are not correct in believing that, in all cases, the male hormone is stimulating in effect, and never inhibiting. Not only is it inhibiting in action, but even actively antagonistic to the secondary sex-cords already developed to a considerable degree in an embryonic ovary.

There is no evidence found in the pursuance of this study of the formation in cattle of reciprocals of the free-martin as inferred by Hartman ('20) for the Opossum and evidently accepted by him as possible in Man and Dog.

MATERIAL AND METHODS

All the embryos upon which this study is based were collected by the writer on the beef-killing floor of Swift and Company, in Chicago, between April, 1921 and April, 1923, and the writer is indebted to them, and to James Walker, their foreman on the killing floor, for kind cooperation and valuable assistance.

Uteri were opened as soon as taken from the cow, about 20-25 minutes after her throat had been cut, the chorions taken out and opened, and the embryos less than 30. millimeters in length, killed and fixed, entire, in Bouin's fluid. Sizes up to 8.0 cm. forehead-

anal length had the abdomen opened, the liver and intestines taken out, and were then immediately put into Bouin's fluid. Larger sizes had the front quarters cut off at the umbilicus level, the symphysis pubis and ischii cut through, and spread, to let in the fluid, and were then put in Bouin's fluid. This insured killing and fixing fresh, for, in some cases, embryos of 4-6 cm. had the heart continue to beat for from 12-28 minutes after their thoraxes were opened. In no case has material, that was kept more than two hours before going into the Bouin's fluid, been used in this study.

After 24-36 hours in Bouin's fluid kept strong by changes into new fluid, the material was washed in 50% and 70% alcohol and then in 80% alcohol to which a trace of Lithium carbonate had been added, for two days to remove Picric acid. It was then kept in 80% alcohol till dissected for drawing or photographing before staining and sectioning. After drawing, for record of gross anatomy, the larger material, 7.5 cm. and over, had the right gonad and ducts back to the union of the genital cords, cut off and embedded separately for section. The pieces so trimmed were stained in bulk in Alum Cochineal for 24-30 hours, then dehydrated in alcohols and synthetic oil of Wintergreen and embedded in soft paraffin for serial-sectioning from 15-40 micra thick, except in cases where the other side was sectioned thinner for comparison. After mounting, the section series were counterstained in Orange G, or Lichtgrün, but the latter stain was abandoned, as it did not give as clear differentiation as the Orange G.

Measurements as given in this study are forehead-anal lengths, for those that had straightened out, and from the cervical flexure to the opposite curve of the rump, in smaller ones. These measures were easiest to make accurately at the time of collection on the killing-floor, in the cases of the larger ones, and for the smaller ones, where measurement could not be accurate till killed and fixed,

because unfixed tissues were too nearly transparent, it was the most constant length easy to measure accurately. If very young stages are to be studied some adjustments will be necessary.

In all cases except those of the 15.0 cm. female (Fig. 9) and 20.3 cm. male (Fig. 19) the left set of ducts and the parts common to both sides were measured for the reconstructions, but in those cases the right side was substituted because of accident to the left gonad and genital cord. This makes these reconstructions longer, because the right side has a less advanced stage of retreat of the organs, together with its other evidences of slower development. These facts are stated in connection with the affected parts in the description of the findings.

The illustrations for this paper are made by Mr. Kenji Toda, Figures 1 and 2 from the sections, and the rest from the original drawings to scale by the author.

In making the original drawings, for larger specimens than 3.7 cm., the rectum was taken as reference line, from which to measure the dorso-ventral displacement in micra, as shown by an ocular micrometer calibrated against a Zeiss stage micrometer, each division of the ocular micrometer corresponding to 23.7 micra. Length along the antero-posterior axis was obtained by counting the serial sections from the posterior tip of the phallus forward, and multiplying by the thickness in micra per section. Drawings from 7.1 cm. specimens up, were made to a scale of five in both dimensions, with the exception of the 11.1 cm. male, which, with the smaller specimens, was made to a scale of ten. Lateral displacements are not taken into account, except in the case of the 20.6 cm. female, where the Wolffian duct derivatives are placed ventral to the vagina while they are really in the ventro-lateral wall of that organ. In the case of the guber-

naculum there is some convention used as to the saccus vaginalis and the ligament, to show the cavity in the saccus surrounding the gubernaculum ligament. In such reconstruction it was impossible to show how they joined the cord in either males or females. In all cases the size relations of these structures are to the same scale, and distortions due to faulty position in reagents are not corrected. In cases where a part of a structure is dissected away or injured by accident that structure is shown incomplete. All reconstruction figures, except those of the 16.3 cm. female and 20.3 cm. male, represent mirror images of the organs, as to lateral displacement, so that the organs of the left side appear beyond the ducts whereas they are really nearer and would cover them. The ducts are represented as cut by median vertical longitudinal section through their entire length, but in cases where the lumen was less in diameter than the thickness of a line at the scale of the original drawing the duct appears solid. So the drawings must not be taken as an absolute guide as to presence or absence of lumen.

In the case of embryos less than 6.1 cm. forehead-anal length the notochord was used as reference line, in the anterior part of each drawing, because the rectum makes a ventral loop near the posterior end of the mesonephros. Some slight distortions may be due to twisting of sections a little in mounting and must be taken into consideration in a critical examination of the figures.

TABLE 1.

List of Figures and Animals for Study.

Fig. No.	Length of embryo	Sex	Side used	Structure shown.
1	2.3 cm.	Female	Left	Section gonad and ducts
2	9.0 "	Free-martin	"	"
3	2.2 "	Female	"	Reconstruction
4	3.0 "	"	"	"
5	5.4 "	"	"	"
6	6.1 "	"	"	"
7	7.5 "	"	"	"
8	9.0 "	"	"	"
9	13.0 "	"	Right	"
10	19.5 "	"	Left	"
11	20.3 "	"	"	"
12	2.8 "	Male	"	"
13	3.0 "	"	"	"
14	5.5 "	"	"	"
15	6.1 "	"	"	"
16	7.1 "	"	"	"
17	10.3 "	"	"	"
18	17.0 "	"	"	"
19	30.0 "	"	Right	"
20	9.0 "	Free-martin	Left	"
21	10.3 "	" [illegible] 2	"	"
22	11.75 "	"	"	"
23	17.0 "	"	"	"
24	20.0 "	"	"	"

Animals Studied, but not Figured

0.8 cm.	Indifferent	Both sides	"
1.2 "	"	"	"
1.4 "	"	"	"
1.6 "	"	"	"
1.7 "	"	"	"
1.9 "	"	"	
2.4 "	"	"	"
2.5 "	Male (presumed)		"
2.7 "	" (certain)	"	"
15.0 "	"	Left	
20.5 "	Free-martin	Gross Anatomy	

DEVELOPMENT OF ORGANS AND SYSTEMS.

The Gonad.

Since the gonad of the Free-martin has been studied by Chapin ('17) and Willier ('21), and is not the main topic of this study, it is introduced here mainly as a check on the other work. It is partly confirmatory of previous results and in addition introduces some new material.

In the indifferent stages;- at 0.9 cm. there is no sign of the genital ridge, at 1.2 cm. the genital ridges just start to thicken on the medial aspect of the mesonephros. They are quite marked at 1.4 cm. as well defined ridges on the ventro-medial aspect of the mesonephros connected to it by mesenteries. No sexual differences are visible.

In females;- At 2.8 cm. the male and female gonads can be distinguished. The ovary has a narrow tunica albuginea separating the primary sex-cords from the germinal epithelium which is several layers thick at this stage. From this stage upward the sex can be distinguished by the gonad sections under a 16 mm. objective.

At 3.8 cm. the germinal epithelium is four to six or even more cell-layers thick. The primary tunica albuginea as described by Chapin ('17) and Willier ('21), is more evident.

5.1 cm. females have the germinal epithelium proliferating inwards and producing the region of secondary sex-cords or cords of Pflüger, which are cut off from the medulla of the ovary by the less intensely stained primary tunica albuginea. The medulla contains in its margin next the mesentery a definite rete.

The ovaries of 7.5 cm. embryos are decidedly female in type with a cortical layer consisting of several layered germinal epithelium and secondary sex-cords, outside and separated by the primary tunica albuginea from a medulla of primary sex-cords and rete. This tunic is becoming vascular. The gonad is of the type described by Chapin ('17) for the normal ovary of this sized embryo.

At 9.0 cm. (Fig. 1), the germinal epithelium is still connected to the thicker layer of secondary sex-cords, the primary tunica albuginea is more vascular, the medulla loosely packed, and the rete remains near the mesentery.

At 16.0 cm., the ovary is larger, but shows the same general topography in section as the 11.0 cm. normal female in Chapin's ('17) paper, except that the definitive ovarian tunica albuginea is not yet forming.

At 20.0 cm. the germinal epithelium is still connected to the secondary sex-cords which are thicker, and the primary tunica albuginea is thicker and more vascular. The rete is near the mesentery in position.

Particulars as to the later stages are given by Chapin ('17) and are beyond the scope of this paper.

In males;-

Testes are first distinguishable from ovaries at about 2.5 cm. by the fact that the germinal epithelium becomes but one layer thick and the cells, just below it, differentiate into a lighter staining layer, the tunica albuginea, enclosing a denser staining medulla, of cells which will later give rise to the primary sex-cords and finally to the seminiferous tubules. The rete is more centrally placed than in the female, but is hardly to be distinguished from the surrounding cells at this stage.

At 2.5 cm. the cutting off of the germinal epithelium from the sex-cords is more marked. The tunica albuginea is wider and stains less darkly than in the female. The rete is nearer the center of the gonad and is more clearly distinguishable from the sex-cord region of the medulla than in the female.

At 3.8 cm. the serous epithelium of the gonad is one cell thick and its cells are flattened. The tunica albuginea is crowded out to a thin layer near the periphery of the gonad by the larger sex-cord region. The rete is central and darker stained than in the female, where also the tunic is not so well-defined.

At 5.1 cm. the sex-cords are growing outward and form a definite layer surrounded by tunica albuginea. The sex-cords are organizing into tubules next the tunica. No such definite arrangement is evident in the female.

At 7.4 cm. the tunica albuginea of the testis has become more vascular in its inner part next the sex-cords and begins to form the tunica vasculosa, making the whole tunic appear thicker. The rete is central.

At 10.9 cm. increased size is the only apparent change.

At 17.0 cm. there are definite tubules extending from the tunica vasculosa to the rete. These have primordial germ cells, each with a large nucleus surrounded by a clear zone. This gonad resembles Chapin's figure of the testis of a 24.0 cm. normal, and Hillier's for a 20.0 cm. one, as does the one here studied.

The Free-martin Gonad.

Lillie ('17) describes the gonad of a 3.75 cm. free-martin and Chapin ('17) one of 7.5 cm. and the reader is referred to their figures for these stages.

In 9.4 cm. free-martin No. I (Fig. 6), the gonad is but

slightly smaller than that of the normal female, which it resembles in many respects as to cross-section. However the secondary sex-cords, or cords of Pflüger, are already cut off from the germinal epithelium by a secondary tunica albuginea of male type, and the germinal epithelium has become flattened. This tunica is approximately like that of a male of 3.8 cm. and no tunica vasculosa is evident. The secondary sex-cord zone is almost as thick as in the normal female, but is not so much invaded by blood-vessels from the vascular primary tunica albuginea and medulla. The cells on its outer margin are heavily pigmented, and appear to be undergoing degeneration in a centripetal direction. Inside this zone is the vascular primary tunica albuginea like that of the normal female, but not nearly so broad nor so loosely constructed. Inside this tunic is the primary sex-cord region, more dense than in the female, but without organization into tubules, and resembling that of a younger male. The rete is located near the mesentery as in the female.

Such an extensive development of secondary sex-cords has not been described hitherto for foetal free-martins, and Chapin ('17) says definitely that secondary sex-cords never occur in free-martins. This case presents a great contrast to the normal female in which the secondary tunica albuginea is lacking up to 10.0 cm. That this female is undergoing modification in the male direction is also substantiated by the condition of the ducts, for the Müllerian duct in the section adjacent to the gonad is reduced to a mere vestige while the Wolffian duct appears to be larger and is certainly more highly organized than in the female (Figures 1 and 2).

Willier ('21), in his discussion of the possible origins of some of the follicles in the sex-cord region of his adult free-martin

'17, mentions the possibility that they may be derived from cords of Pflüger that invaginated before the sex-hormone began to take effect or else from the medullary follicles of the ovary. The present case shows that the former supposition may be well-founded, and presents an interesting exception to cases hitherto described. The effect of the modifying agent dates from a time after the secondary sex-cords were formed in the female. When the twins, to which this free-martin belonged, were collected, it was recorded that the fusion of the chorions was very narrow, and the vascular connection between them was slight. The bull twin was 9.7 cm. in length. The gonad condition taken with the small connection between the chorions would suggest late chorionic fusion.

In 9.3 cm. free-martin No. 5, the gonad is essentially like those figured by Chapin ('17) for her 10.5 cm. free-martin. There is a single layer of epithelial cells covering the gonad under which is a thin tunica albuginea. This secondary tunica is separated from the primary one in two regions, by groups of cells the exact nature of which the thickness of the sections made it impossible to determine. The rete is located in the centre of the medulla. The Müllerian duct is vestigial, in the ridge on the mesonephros adjacent to the gonad, while the wolffian duct is better developed than in the normal female of the same size.

In an 11.75 cm. free-martin, twin to a 14.0 cm. male, and having its head and shoulders as large as those of its twin brother, but hind quarters much smaller and comparable to those of a 7 or 8 cm. embryo, the gonad is much reduced in size, and has attachments and ducts like those of a male. Sections show two distinct regions, one next the mesentery like the rete region described by Chapin for

her 16.3 cm. free-martin, and another similar to the sex-cord region, but little differentiated into sex-cords. It is not clear whether this is a secondary sex-cord region or a part of the medulla of the original ovary; but, in any case, it is definitely separated from the rete region by what resembles a definite tunica albuginea. It is not so definitely separated from the germinal epithelium as in the rest of the gonad, but it is nevertheless separated from it by a tunic similar to that of a very young male about 3.8 cm. The epithelium covering the gonad is flattened and appears to be only one layer thick. The rete has a more definitely organized region near its centre with less dense tubules around it.

The gonad has a primitive epididymis-like connection at its front end, like that in the male, and an appendix testis or sessile hydatid of Morgagni as a remnant of the ostium tubae abdominale and fimbriae of the ostium, which has some connection forward to the rete of the gonad in the mesentery anterior to the gonad itself.

The gonad of the 17.4 cm. free-martin studied, approximates that of the 3.8 cm. male in size and general appearance, but with less well-defined tunica albuginea. There are no secondary sex-cords. The primary tunica albuginea is thinner than in the 3.8 cm. male. The primary sex-cords are not as well organized as in the 4.5 cm. male. There is a paler-stained zone between the sex-cords and the rete, and it is near this zone that denser growth appears to be, and in it that the vascular supply is found, not in the tunica vasculosa. The rete is larger than in the male and is located near the mesenterial region or hilus, as in the female. The sessile hydatid of Morgagni is present in addition to a coiled Wolffian duct forming an epididymis round the dorso-lateral side of the gonad.

In the 20.0 cm. free-martin studied, the gonad has developed like that of a younger male. It is smaller than that of the 17.4 cm.

free-martin, but the differentiation of the tunica albuginea, sex-cords, and rete, is more definite. The tunica albuginea and sex-cord region are more decidedly male in type and resemble those of a 9.1 cm. male. The rete reaches a more central position than in the female, and connects with the Wolffian duct through the cranial group of mesonephric tubules. The hydatid is not noticeable as in the preceding case and has lost its columnar epithelial coat. The blood vessels are still in the region between the rete and the sex-cords, as in the female, not outside the sex-cords as in the male from 7.8 cm. upwards. A coiled epididymis is present as in smaller males but out of relation to gonad age. It therefore appears that in some cases the cortex of the ovary, as represented by the secondary sex-cords, develops in free-martins before the male hormone begins to be effective, but that, in such cases, the hormone causes degeneration of the cords from their periphery inward, so that they are not found in the later stages as a rule. But secondary sex-cords do not appear at all in other cases as shown by Lillie ('17) for a 3.75 cm. free-martin. In all cases so far recorded the germinal epithelium is cut off from the sex-cords by a secondary tunica albuginea like that of a male of a younger stage, if not by the primary one as in the male. This indicates a considerable amount of variation in secondary sex-cords in the free-martin. In four out of the five studied there is no definite evidence that these have ever formed. But in the fifth case (8.8 cm. free-martin # 1, fig. 23) the cords were highly developed and undergoing secondary degeneration. This is correlated with reduced size of free-martin gonads as compared with normal males or females in early stages, later sometimes followed by increase in size far beyond that of the normal ovary, and assumption of testis characters with smaller size than the normal testis.

The Wolffian Ducts and Their Derivatives

The origin of the ducts in both males and females can be taken for granted so far as the scope of this paper is concerned. They have already reached the sinus urogenitalis in the 0.7 cm. embryo studied, at which stage the ureters are easily seen as dorsal diverticula from the Wolffian ducts, near the sinus, as described in the text books for the Pig and other mammals. The rectum also enters the sinus a little posterior to the point of entry of the Wolffian ducts, but at 2.4 cm. a short perineum is present, having begun to form at approximately 1.7 cm.

No sexual differences in the Wolffian ducts proper are evident up to 3.1 cm. At 1.8 cm. the ureters are still joined to the Wolffian ducts, and at 2.4 cm. they come off these ducts just where they open into the sinus, at which time the ureters pass laterally, then forward from their point of entry, and dorsally, and finally join the central duct in the kidney; at 3.7 cm., in the female they have separated from the Wolffian ducts, and enter the sinus farther forward, on a ridge in the dorsal sinus wall. Their course to the kidney will not be traced further but their point of entry into the sinus will be noted as a sexual difference. In the 5.4 cm. male (Fig. 12) they come off the sinus laterally where the ducts open to it. In the female the part of the sinus between the openings of the Wolffian ducts and those of the ureters lengthens rapidly, separating the two sets of openings farther and farther. This takes place much more slowly in the male, so that in the female at 9.3 cm. (Fig. 8) the distance is nearly double what it is in the 10.3 cm. male (Fig. 17), a difference which persists to the 20.5 cm. stage, which is as far as this investigation is carried (Figures 11 and 19).

In 9.0 cm. free-martin No. 1 (Fig. 22), this distance is as long as, or longer than in the female. In No. 2 of the same size (Fig. 23), it is shorter than in the female, and in that case the Müllerian ducts are carried farther back than in No. 1 or in the female before uniting with the sinus. At 17.0 cm. (Fig. 25), the relative distances are :- female, 3.4: free-martin, 4.1: male, 1.9: and at 25.0 cm. (Fig. 26) they are;- female, 4.5; free-martin, 4.4; male, 2.4. The strip along which this variation occurs is mesodermal in origin, while the rest of the sinus is entodermal.

Wolffian ducts proper in the female.

At 6.1 cm. (Fig. 6), the Wolffian ducts show evidence of retrogression by a slight narrowing at the level of the posterior end of the uterus where the Müllerian ducts are separate. No seminal vesicle primordia are to be seen.

At 7.4 cm. (Fig. 7), the Wolffian ducts of the female have diminished in cross-section throughout, but a single slight lateral outpouching on each side is seen in the region beside the vagina, about 60-70 micra long, the homologue of the seminal vesicle. The Wolffian ducts are continuous throughout, from the anterior end of the mesonephros, where they receive the collecting tubules of that organ and are indistinguishable from them, to the sinus. Tubules of the mesonephros enter the ducts throughout the whole length of that organ.

At 9.0 cm. (Fig. 8), the female shows further reduction of the Wolffian ducts, but they are still continuous, though smaller than the Müllerian ducts throughout. Seminal vesicles are present as two small separate lateral diverticula from each Wolffian duct about 100 micra apart. The left duct is the larger in my specimen. From this size upward the ducts and vesicles of the left side seem

to be in advance of those of the right side in development. The Wolffian ducts are relatively so much reduced, that they are incorporated into the accessory layers of the walls of the Müllerian ducts, lateral and somewhat ventral to them.

In the 15.3 cm. (Fig. 9) female, the Wolffian duct is present anterior to the gonad in the region of the cranial group of tubules, but has lost all connection to the gonad. It is indistinguishable from the tubules in appearance. It has completely disappeared in the region of the gonad and as far posterior as the ventral loop of the uterus, where it is present as a solid cord and then intermittent and very faint in section for a short distance. It is entirely absent in the region of the body of the uterus, and in the cervix uteri region, it is represented by three small vesicles, separated from each other and dorso-lateral to the cervix. These are the homologues of the seminal vesicles. Posterior to these the duct is absent for a considerable distance. From about the anterior quarter of the vagina, the duct is continuous to its opening into the sinus. In some places it has a lumen, but it is solid for the greater part to the place where the vagina begins to narrow. From there on it has a lumen increasing in size.

At 16.5 cm. (Fig. 10) also, the Wolffian ducts are intermittent. They are entirely absent in the region of the cervix and body of the uterus, in some places, while in that region the seminal vesicle homologues appear as outpouchings from the ducts, where they are present. The ducts are continuous from the anterior end of the mesonephros back to the body of the uterus. The right duct is more degenerate than the left. A lumen is present only in scattered places in front of the break in the ducts, and opposite the posterior half of the vagina.

At 23.9 cm. (Fig. 11) in the female, the Wolffian ducts are gone entirely in the gonad region and parts anterior to it, and even the cranial group of mesonephric tubules is almost gone and nearly

indicating [illegible] as such. The duct first appears in the ventral wall of the uterine horns at the places where they bend ventrally and back toward the body of the uterus, as a solid cord which reaches back to the level of the union of the horns. A small piece of it remains open on the side of the cervix uteri and has an outpouching off it, as a seminal vesicle homologue. Some distance farther back, on the ventro-lateral wall of the vagina, another piece of the duct and another seminal vesicle homologue appear, and about the middle ofthe vagina, a third occurs. These three are not connected at this stage nor are they connected to the other duct remnants. Posterior to a short space behind the last mentioned remnant, the duct becomes continuous, but gives off at least two more little side pouches. The duct is solid back to the posterior fifth of the vagina, but has an enlarging lumen behind that, and opens into the ventral side of the vagina near the vestibule.

There are present in the female four or five vestigial homologues of seminal vesicles, none of which could be seen in gross dissection. The ducts, where present, are found in the ventral and lateral walls of the vagina and uterus in the older stages.

Wolffian ducts in the male.

The Wolffian ducts are the same in males and females up to 4.8 cm., but at 6.1 cm. they are larger in lumen in males. Primordia of seminal vesicles, one on each side, appear at this stage, as lateral diverticula from the duct. (Fig. 15) In this region the ducts are larger than in the female. (Fig. 5).

At 7.3 cm. (Fig. 16), the ducts are still developing, while in females they are not (Fig. 7). In the male the seminal vesicles are progressing and grow out laterally, then dorsally and anteriorly, but unbranched as yet, and one on each side. The Wolffian ducts open

separately to the sinus, as in the female.

At 16.3 cm. (Fig. 17), the male has Wolffian ducts with continuous lumen of somewhat variable diameter at different levels. The seminal vesicles do not branch, but their lumen gets much larger than that of the ducts adjacent to them, and they develop a thick wall lateral and slightly dorsal to that of the ducts. The ducts open to the sinus slightly cranial to the vaginal opening. The connection of the rete of the testis to the Wolffian duct of each side through the cranial group of mesonephric tubules is seen in the epididymis, which is recognizable as such.

At 17.9 cm. (Fig. 18) the Wolffian ducts in the male have lumina throughout; the rete of the testis connects the tubules of that organ with the cranial group of mesonephric tubules, and thus to the Wolffian duct; and the genital cord, containing the duct, has developed a backward loop in the region where the gubernaculum attaches to it. In the male the Wolffian ducts take most part in the loop, while in the female the Müllerian ducts take most part. In the male at this stage the Wolffian ducts occupy most of the cord since the Müllerian duct derivatives are almost gone. In males the seminal vesicles are large and branched, but have a single duct leading to the Wolffian duct on each side, into which each opens, actually, as well as relatively, nearer the openings of these ducts into the sinus than in earlier stages. The female at this stage has at least two or three small primordia of such vesicles on each side and much farther forward, in the region where the ducts are interrupted.

At 22.1 cm. (Fig. 19), the changes in the Wolffian ducts recorded above for the 17.9 cm. male are more accentuated. The backward loop of the genital cord is more marked and the duct participates in it. Seminal vesicles are larger and more branched, and their single ducts extend back farther and open into the Wolffian ducts as in the

17.0 cm. embryo. Prostatic vesicles surround the posterior ends of the Wolffian ducts and seminal vesicle ducts.

In only one case in all the males studied, in contrast to the females, was there any sign of more than one primordium of seminal vesicles on each side, and then only a very minute side pocket off the Wolffian duct just behind the main one. It did not show any sign of developing into a duct or vesicle.

Wolffian ducts in the free-martin.

In the 9.0 cm. free-martin No. 1 (Fig. 20), the least modified one, the Wolffian ducts are continuous throughout, as in the male. In the genital cord they bear the same relation to the Müllerian ducts as in the male, being larger than the Müllerian ducts in the anterior part, but smaller in the posterior part, and in the uterus and vagina regions. The seminal vesicles are single and unbranched as in the male, but of only about one-third the length, and smaller in diameter than in the male. They barely reach the same size of lumen as the small part of the Wolffian ducts adjacent to them. This free-martin is much nearer to the female type in size relations of the Wolffian ducts than to the male, as found in the 10.0 cm. one studied, or even in the 7.5 cm. one. The seminal vesicles are small, but in place of two minute diverticula on each Wolffian duct, as in the female, it has only one as in the male. The connection of the ducts with the tubules of the gonad through the rete and cranial group of mesonephric tubules is retained as in males. The ducts are modified female ducts, and the condition seen here can be easily accounted for in conformity with the interpretation of the gonad in this case (p. 16), by supposing that this female came late under the influence of the male hormone, and had already reached a fairly definite female differentiation before the hormone could cause reaction in the male direction. This male reaction is more evident in

the anterior parts of the ducts, than in the posterior.

9.0 cm. free-martin No. 2 (Fig. 21), which has a more male type of gonad, has its Wolffian ducts also closer to the male type. They are as large as the Müllerian ducts, beside which they lie in the region of the uterus and vagina, excepting the posterior quarter of the vagina which is larger than they. The seminal vesicles are larger and longer than in No. 1, and even show signs of a branching in their anterior ends. They are larger in lumen than the Wolffian ducts in that vicinity, but have only one duct each. The Wolffian ducts are complete and lined with well-defined epithelium throughout even though the lumen is very minute in their anterior ends. In that part of the genital cord anterior to the attachment of the gubernaculum ligament the Müllerian ducts are absent, except for a small piece nearly opposite the centre of the gonad, and a piece near the ostium tubae abdominale, which has not completely degenerated. The Wolffian ducts extend to the forward end of the gonad, where they are connected to the rete of the gonad through the remnants of the cranial group of Wolffian tubules.

In the 11.75 cm. free-martin (Fig. 22), the Wolffian duct on each side extends forward only to the point where the gubernacular ligament joins the genital cord. From this point forward, the cord is present but has no lumen, nor sign of a duct in it. Posterior to this point, there is no lumen in the sense of a definite epithelial lining for a considerable distance, but it is marked in section by an area of degenerating tissue. There are five separate little evaginations of the Wolffian duct in the seminal vesicle region on each side, a female condition, and of these the most anterior one alone attains any considerable length. Of the two ducts, the left is the smaller for some distance anteriorly from their opening to the sinus, and each has a lumen as far forward as the seminal vesicles

extend. The anterior seminal vesicle has a larger lumen than the ducts themselves, and shows some signs of preparing to branch at its anterior end. In this case the Wolffian ducts show little effect of the male hormone, and were presumably so far advanced in female differentiation that they were already degenerated in some regions, before the male hormone started to act. But the development of the anterior seminal vesicle is a male characteristic.

In the 17.0 cm. free-martin (Fig. 23), the Wolffian duct is continuous from the front of the mesonephros to the region of the uterus where it is interrupted in four places. The left is better developed than the right, but both have the interruptions. They are again interrupted in the region of the anterior seminal vesicle, which, though one of four on each side, develops in the true male fashion, and even branches at its anterior end into tubules with a coat distinct from that of the genital cord, but like it. The three posterior diverticula on each side, primordia of accessory seminal vesicles as in females, are more marked than in the 16.5 cm. female (Fig. 20). Of all these seminal vesicles, the anterior one on each side is similar to those of a younger male, and the last three are merely imperfectly stimulated growth centres held over from the female period and not inhibited completely as in the male. The lumen of the posterior part of the Wolffian duct extends forward to where the anterior seminal vesicle comes off on each side. In this free-martin we have again a condition intermediate between that of a female and that of a younger male. The supernumerary seminal vesicles are characteristic of females, whereas the anterior seminal vesicle condition and the continuous anterior end of the duct are distinctly male.

The 20.0 cm. free-martin has the most extreme modification in the male direction of the five studied (Fig. 24). The Wolffian ducts

are continuous throughout their entire length as in a male, though the lumen is not so large nor so well carried forward. The Müllerian ducts are represented only by a short, but patent uterus masculinus in the region where the genital cords separate, but attached more to the right cord than to the left. Lillie figures it on both sides in some of his specimens.

At the front end the ducts extend forward past the place where their last connection to the Wolffian body is complete and branch into minute ducts, that lead to resorbed parts of the Wolffian body. The seminal vesicles are much more highly developed than in the 17.0 cm. free-martin. There are no less than eleven of these on the left side and about as many on the right. Of these the anterior is the longest and thickest, and branches only near its tip. The second is next in length and a little more branched. The third is a little shorter but more branched. They get progressively shorter posteriorly and less branched again till the last two or three are merely short diverticula projecting ventro-laterally and then extending forward along the Wolffian duct. The lumen of the duct narrows just anterior to the opening of the anterior seminal vesicle. The female of this size had only five small outpouchings and they occurred on an interrupted Wolffian duct.

Here again is an evidence of a female condition later modified in the male direction by the male hormone which stimulated the Wolffian duct. The male at this size has but a single seminal vesicle on each side with many branches, all opening into a common duct, that passes back and opens into the Wolffian duct near the sinus. In the female the seminal vesicle homologues are farther from the sinus. In the free-martin this distance is intermediate. In the female and free-martin the Wolffian duct on each side appears to open into the floor of the vagina just before it opens into the sinus,

while in the male it appears to open directly into the sinus from the medial sides of two grooves lateral to a median ridge on the dorsal sinus wall. The free-martin resembles the female more than the male in this respect.

To sum up the Wolffian duct situation it may be said that;- the first sexual difference to appear is in the relation of ureters to the ducts. Up to 2.5 cm. the ureters come off the Wolffian ducts in both sexes. At 2.8 cm. the ureters in the female come off the sinus anterior to the ducts, while in the male they come off with the ducts. The distance between the openings of the ureters and ducts on the sinus wall gets longer in the female than in the male and is twice as long as in the male at about 10.0 cm. This sexual difference is maintained through the 20.0 cm. stage. The free-martin is generally intermediate in condition, but more nearly approximates the female.

The female has a narrowing of the ducts throughout, appearing at about 5.1 cm. and progressing, till, at 15.0 cm. the ducts are interrupted anterior to the vagina region, and entirely absent in the gonad region in some cases, but present and continuous in that region in others. This degeneration proceeds till the ducts are present only in the uterus wall, as solid strips, and in the walls of the vagina, as isolated minute segments in the anterior half and continuous in the other half, with lumen only in the posterior one-third.

In the male, these ducts retain a connection with the tubules of the gonad through the cranial group of mesonephric tubules and rete of the gonad, which is early lost in the female. The ducts continue to develop and to have a lumen to their anterior ends, and occupy the whole genital cord in parts where the Müllerian duct is degenerated.

In the female the manner of opening into the sinus differs from that in the male, in that the ducts join the vagina before opening into the sinus in the later stages, while in the male they open separately

to the sinus.

In free-martins the condition of the ducts is variable. The connection of the gonad tubules to the Wolffian duct is retained in four of the five cases studied, and, in the exception, the whole of the anterior part of the duct was degenerated as in the female. The ducts themselves vary in condition, but, with the exception of the 11.75 cm. free-martin, where they appear to have behaved as a female set for some considerable time and degenerated, then to have been stimulated; they are male in type, and represent those of a male of an earlier stage.

As to the seminal vesicles;- they develop somewhat later in the female, 7.5 cm., while in the male they appear first at 6.1 cm. In the female there are five or perhaps more primordia that begin to develop at various stages, but none reach any size appreciable in gross dissection, and in later stages they appear on an interrupted duct, and sometimes on isolated parts of it, always farther forward than in males in later stages.

In males one vesicle develops and grows strongly to a considerable length before branching, and its single duct opens into the Wolffian duct progressively nearer the posterior end of that duct, as the embryo increases in size. In one case there was a minute second outpouching of the Wolffian duct in the region where a second vesicle would come in a female, but it did not develop.

In free-martins there are as many as eleven such primordia that get a start. They are not so long as in males of corresponding size, but are developed after the male pattern. In this also there is variation in free-martins of the same size. Here also the 11.75 cm. free-martin (Fig. 22), is more like a female than the others and appears to have developed longer as a female. In the larger stages, though more numerous than in the male, they develop like them. The oldest one with its eleven separate vesicles on each side gives a nice picture of a gradient of differential growth and inhibition

along the axis of the Wolffian duct in the seminal vesicle region.

In the free-martin the opening of the ducts into the sinus is sometimes direct, as in the male, and sometimes into the vagina first, as in the female. This bears a relation to the state of other organs in reference to the degree of influence of the male hormone. It is more like the male where the other parts are more affected.

The free-martin Wolffian duct derivatives show progressive modification of a female early differentiation toward a male later differentiation, under the influence of a male hormone. The degree of this modification appears to depend on the time of inception of the hormone and the time elapsed from that inception to the stage studied.

The Müllerian Ducts and Their Derivatives.

In the indifferent stages;- the Müllerian ducts begin to develop at about 1.2 cm. as a slight thickening of the coelom epithelium on the ventro-medial surface of the Wolffian duct ridge, about one-quarter of the distance back from the anterior end of the mesonephros, but no invagination is to be seen. At 1.4 cm. the ostium tubae abdominale and anterior end of the tube have begun to form. The tube develops by an infolding of the coelom wall on the ventro-lateral surface of the mesonephros just ventral to the Wolffian duct. From the first its lining differs from the coelom epithelium and from that of the ostium. The epithelium is columnar, but not so thick as that of the ostium. The ostium lies about one-quarter of the mesonephros length back from its anterior end as Bergschicker ('11) finds in his 1.3 cm. embryo. At 1.[illegible] cm. the duct appears at its posterior end as an infolding or thickening of the epithelial wall of the Wolffian duct, and extends forward between this wall and the coelom epithelium as a distinct cord of a different epithelium, more columnar than either of the others. It has no organized lumen in its posterior end on the

left side, which is in advance of the right in development. The cavity of the ostium extends forward past its opening into the coelomic cavity as a pocket with wrinkled walls and the coelom lining is also thickened for a short distance in that direction. The total length of the duct on the left side is 638 micra, on the right , 374 micra.

At 1.7 cm. the coelom epithelium anterior to the ostium thickens markedly on both sides. Then on tracing the sections caudally, a fimbriated slit from the coelomic epithelium leads into the interior of the flat duct, at about the same level on both sides, lined with taller columnar cells and lying ventromedial to the Wolffian duct. This ends posteriorly as before in a cord on the ventral wall of the Wolffian duct. Left side is 553 micra and the right is 765 micra long.

At 2.0 cm. the ostia are attached to the diaphragm and the ducts have lumina for some distance, the left being greater as before.

At 2.4, 2.5, and 2.7 cm. the ducts show increase in length in which the left is in advance of the right. No such difference between the sexes in the matter of one or two ridges for the Wolffian and Müllerian ducts, as is described by Felix ('12) for the human embryo, is found in this series for cattle. The ostium is farther forward on the right side than on the left due to the backward migration of the mesonephros as described by Bergschicker ('12). The ducts do not reach the sinus in these stages.

In females:- At 2.8 cm. (Fig. 3) neither right nor left duct has reached the sinus though the left has got as far back as the opening of the Wolffian duct to it. Lumina are present throughout their length. No separate Müllerian duct ridge is present in the female of this stage.

At 3.0 cm. (Fig. 4)in females, the ducts reach the sinus posterior to the Wolffian ducts, but have not yet united to form uterus or vagina. The epithelium round the ostium is medium columnar.

At 3.8 cm. (Fig. 5), the ostium epithelium extends from the Müllerian duct to the rete of the gonad, where it reaches the coelom surface, after joining a glomerulus of the Wolffian body. The Müllerian ducts of the two sides come together in the median plane, but do not fuse to a single lumen, and separate posteriorly before they open to the sinus on the medial sides of the dorso-lateral grooves, along which they extend for a distance before their lining disappears.

At 5.1 cm. (Fig. 6) there is a slight epithelial, but not tubular, connection of the ostium, with its tall columnar epithelium, to the rete of the gonad and a glomerulus of the mesonephros. The Müllerian ducts have lumina throughout but smaller than the Wolffians, except at the anterior end. Posterior to the point of attachment of the round ligament the ducts enlarge greatly to the horns of the uterus, and are four to six times as large as in the maleof the same size. They are beginning to have the transverse portion more marked and the left has more backward migration than the right. They unite to a single lumen in the uterus region, then are double again, and again single in the vagina region. The Wolffian ducts join both the vagina and the sinus at about the same level, but the vagina projects backward from this point of junction as two bays with a tongue of tissue between them and join the sinus separately from each other but not separate from the Wolffian ducts.

At 7.5 cm. (Fig. 7) the ostia are joined as above to the rete of the gonads as well as to the Müllerian ducts. The left ostium is farther posterior to the right than before. The rete connection is much smaller than before, and the ostium lining is much taller than in preceding

females. The ostium is now as far forward as the tubules of the mesonephros, and the Müllerian duct extends farther forward than the Wolffian one, which is represented by a tubule in the degenerating mesonephros. In the gonad region the Müllerian duct is three times the size of the degenerating Wolffian duct, and is actively developing. The uterus horns are relatively larger and the corpus uteri and vagina are formed as single tubes with double regions between them. As judged by the epithelial lining the Müllerian ducts join the sinus behind and separate from the Wolffian ones.

At 9.0 cm. (Fig. 8), the ostium has turned to face the gonad anterior end, and has a partition down its middle, and a ciliated lining. The details are well described by Bergschicker ('12) and will not be gone into here except as they relate to sex differences.

At 15.0 cm. (Fig. 9), the infundibulum is seen as a little side tube off the ostium, to the rete and thus to the gonad, but it does not connect to the sex-cords of the gonad as in the male. The whole connection is the epoophoron, parovarium or organ of Rosenmuller, as contrasted with the ductuli efferentes of the male. The coil of the uterine horns is becoming more accentuated by reason of their own growth, not from a pull by the round ligament, which is attached half way down one side of the coil not at its tip. The annular folds at the cervix appear and the vagina, with its smooth lining in contrast to the wrinkled lining of the uterus, enlarges greatly. In its walls are found the remnants of the seminal vesicle homologues or Gartner's ducts, together with the Wolffian ducts themselves. The posterior end of the vagina is filled with epithelium, and the Wolffian ducts join it, and a groove from the vestibule meets them medially, instead of two grooves as in earlier stages. The vagina extends posteriorly as a dorsal corridor of the sinus.

The 16.5 cm. (Fig. 10) stage shows only an enlarged vagina, and at 20.0 cm. (Fig. 11), the whole genital cord is filled by the Fallopian tubes and uterine horns and the coil of the uterus is complete, apparently as in the adult. The rete still joins the posterior end of the ostium. The vagina has enlarged tremendously in lumen, but not in wall thickness. The vestiges of the seminal vesicle homologues are in the thick walls of the cervix uteri and anterior part of the vagina. The dual origin of the vagina is still evident at its posterior end where it is still divided into two backward projecting bays in its dorsal part while the ventral part opens to the vestibule as a single tube after receiving the Wolffian ducts as before described.

In males:- The Müllerian ducts are shorter in males, smaller than 2.8 cm. (Fig. 12) than, in females of like size and do not yet reach the sinus. There seems to be a sexual difference in the rate of growth or formation of these ducts even in stages that appear sexually indifferent externally and in which there are no interstitial cells in either sex. (Lillie, '17, '23; Bascom, '23)

The ducts reach the sinus by the 3.0 cm. (Fig. 13) stage and are in all respects like the female ones except for want of lumen in the anterior and extreme posterior ends, and the fact that they appear to open to the Wolffian ducts where they are open to the sinus.

The ducts are solid in many places by the 5.1 cm. (Fig. 15) stage and show signs of degeneration. The connection of the rete testis to the ostium is larger than in the female. The remnant of the duct is in a secondary ridge on the genital cord. The ducts fuse to a single uterus masculinus but smaller than the female uterus. They are double in the cervix region and single in the vagina region back nearly to the sinus, where they become double, and join the corresponding Wolffian ducts, then both join the sinus

round the tongue of tissue, as in the female, but the Müllerian ducts extend farther back than the Wolffians.

By 7.5 cm. (Fig. 16) the ducts are discontinuous, and the ostium has lost its thick lining of columnar epithelium, but the rete testis connects to it. The hydatid of Morgagni is present as a small knob on the side of the ostium. The lips of the groove that fuse in the female to give the Mullerian duct, have not fused in this specimen and a slit takes the place of the duct without the characteristic lining. Small remnants of the degenerated duct are scattered along the gonad region in strong contrast to the female condition where it is actively developing. Uterus masculinus is still found. Cervix is solid, but a lumen reappears once and is gone before the vagina proper opens out with its characteristic lining. This divides to two before opening to the sinus as in earlier stages, but here prostates are visible in section near it.

By the 10.0 cm. stage (Fig. 17), the ostium is practically gone as such and the ducts are gone back to the gubernacular attachment to the genital cords, where the right is found much better preserved than the left, and has a lumen first where the cords unite. The Uterus masculinus is about the size of one of the Wolffian ducts. The Müllerian derivatives are absent altogether from the uterus masculinus to the place where the Wolffian ducts nearly reach the sinus, where the Vagina masculina appears, almost filled with epithelium, and divides to two, and these open to the sinus, as in earlier stages.

At 17.0 cm. (Fig. 18) the Müllerian duct derivatives occur only as the minute hydatid of Morgagni, Uterus masculinus with short, but wide lumen, and apparently a derivative of the left duct, and Vagina masculina, with large lumen near the sinus, and surrounded by prostatic tubules. Its lining is not that of the normal vagina nor

is there any opening or connection to either Wolffian ducts or sinus.

At 20.0 cm. (Fig. 19), no vestiges of the Müllerian ducts were found anterior to the region of union of the genital cords where an unpaired solid body extends back to the seminal vesicle region, dorsal to the Wolffian ducts. In the region of the opening of the Wolffian ducts to the sinus the vagina masculina appears as the remnant of the right Müllerian duct, surrounded by prostatic tubules, and connecting with the dorsal wall of the right Wolffian duct, posterior to its opening to the sinus. It is solidly filled with epithelium.

In the free-martin:-

For brevity the following terms will be used for the different parts of the Müllerian duct derivatives;

Ostium fimbriae, for the funnel-like anterior opening to the coelom when more like the female condition, and *Hydatid of Morgagni*, when like the male.

Tubal portion, for that part that corresponds to the *Oviducts* or *Fallopian tubes*.

Cornua uteri for the parts that correspond to the enlarged transverse cornua of the female.

Corpus uteri, for the part corresponding to the body of the adult uterus.

Uterus masculimus, where a derivative is partly referable to both of the above, but not definitely to either.

Cervix uteri, for the region of narrowing just behind the corpus.

Vagina, for the part corresponding to the adult vagina in the female.

Vagina masculina, for a condition that approaches that in the male where only the posterior ends of the tubes are present.

In the 9.0 cm. free-martin No. 1 (Fig. 20), the least modified of all those studied, the duct is intermittant in the tubal portion, and continuous behind the mesonephros, and resembles a stage inter-

mediate between males of 6.3 and 7.5 cm., being less continuous than the former and more so than the latter. The ostium consists of a small knob or ridge covered with columnar epithelium and a slit into the mesonephros anterior to the gonad. This retains more of the columnar type of epithelium than the male, but does not close in to form a Müllerian duct nor turn to face the gonad as in the female. However there are signs further posteriorly that it did close in or grow backward, and later degenerated, in the shape of a degeneration spot at the base of a very small secondary ridge on the Wolffian duct ridge. The Müllerian duct has no trace of an anterior opening to the coelom found in the female, but near the posterior end of the gonad it has a lumen quite as large as the Wolffian duct there. The cornua utori have enlarged till larger than the Wolffian ducts there, but where the genital cords unite the Müllerians are much smaller than in the female, though forming a single corpus uteri, more dorsal in position than in the female. The cervix is single, whereas in the female it is double, and it is single throughout the vagina region. The vagina joins the sinus round the same tongue of tissue described for female specimens, together with the Wolffian ducts, but the vagina lining does not become included in the sinus till posterior to the Wolffian ducts; this free-martin would appear to have had its Müllerian duct derivatives developed to a fairly advanced stage before degeneration was induced.

The 9.0 cm. free-martin No. 2 (Fig. 21), with a much reduced gonad as compared to No. 1, has almost as little ostium development as the 10.0 cm. male (Fig. 17). The Müllerian duct is absent in front of the middle of the gonad, at which point it appears, enlarges, gets a lumen for about 95 micra, becomes solid and disappears not to reappear till near the junction of the round ligament or gubernaculum to the genital cord. In this it closely resembles the condition of

the Müllerian duct in the male between 7.5 and 10.0 cm. From its point of reappearance it is more continuous than in the 7.5 cm. male, a contrast to its condition anterior to that point. It is present farther forward on the right side than on the left, and has a lumen on that side before the left appears. The cornua unite to form a single corpus uteri, which constricts to a cervix, about one-third the size in lumen of the corpus, and then, as the bagina, dorsal to the Wolffian ducts, it enlarges greatly and flattens out laterally. Posteriorly it fills with epithelium and joins the sinus, as in preceding cases, but becomes continuous with the sinus as two grooves in the dorsal sinus wall.

In the 11.75 cm. free-martin (Fig. 22) both Wolffian and Müllerian ducts are discontinuous. The rete connects with the coelom as in the female, and a hydatid appears at the end of it as in the male. No Müllerian duct is found anterior to the cornua uteri and then only in part of the left side, very faint and intermittent. The genital cords are much reduced and the Müllerian ducts are both absent, just in front of the union of these cords. A spherical corpus uteri makes its appearance for 200 micra, with a wide lumen, as a remnant of the left Müllerian duct. A vagina masculina appears, both in the middle vagina region, and in the posterior vagina region, where it has a wide lumen, but fills with epithelium just anterior to where it joins the sinus, after receiving the openings of the Wolffian ducts round the customary tongue of tissue. In this region this embryo is decidedly female; in the anterior regions neither male norofemale. It appears that the differentiation in the male direction had not started till after the Wolffian ducts had degenerated very decidedly, but had started soon enough, and acted long enough to cause very great degeneration of the Müllerian ducts after they had reached a considerable development.

In the 17.6 cm. free-martin studied (Fig. 23), the Müllerian

duct is discontinuous at the anterior end. It appears for about 160-200 micra as a solid plug in the mesentery beside the coiled Wolffian duct that forms the epididymis in this specimen, and also as a hydatid of Morgagni more definite than in the male of this size. The cornus uteri of the left side appears at the attachment of the gubernaculum, but does not extend forward into the lateral part of the transverse portion of the cord, but only backward, to join the larger right horn to form the corpus uteri with its large lumen. The cervix is solid or interrupted, and the vagina is patent in the anterior part, then filled with epithelium, but patent again for a short distance in the posterior region, then again filled with epithelium. It is very evidently degenerating throughout. It receives the Wolffian ducts before opening to the sinus as in females, by a dorsal groove in the median dorsal sinus wall. Thereare no prostates here as in the male.

In the 20.0 cm. free-martin (Fig. 24), the ostium has failed to close in and is not found. The condition there is like that in a male between 7.5 and 10.0 cm. and no hydatid is found. No Müllerian duct derivative is found in the tubal or cornua regions, but its former presence is indicated by a small ridge on the mesonephric ridge. A remnant of the corpus uteri persists as a patent blind tube or vesicle in the wall ofthe right genital cord just anterior to the union of the cords. The vagina is like that of a 15.0 cm. male, sectioned, but not figured for this paper, but a little more degenerated in the middle region, and relatively slightly larger in the posterior region. There is no sign of cervix uteri nor anterior vagina region. The posterior vagina region is much larger than in the male, and receives the Wolffian ducts before it opens to the sinus as in the female. The lumen is wide anteriorly, but filled with epithelium posteriorly. No prostates are seen around it.

Discussion.

In the case of the Müllerian duct derivatives, the conditions

found in the free-martin are easily correlated and explained, if the Müllerian ducts carried on as female till they had a good start in development, then became gradually inhibited and resorbed, under the influence of the male hormone. At the same time the hormone stimulated the active development of the Wolffian duct derivatives, as shown in the increased length and size of the Wolffian ducts and seminal vesicles, a male character, in the lengthening of the distance on the sinus between ureter openings and those of Wolffian ducts, a female character, coupled with several seminal vesicle primordia as in the female, activated to produce enlarged vesicles comparable to those of the male. If, as is assumed here, the male and female conditions are modifications of a common set of Wolffian and Müllerian duct and gonad primordia, in two different directions, by zygotic influences in the female, and by zygotic influences increased by hormone action, in the male, as shown by the above details; then the free-martin, judged by the same criteria, is a female, developed normally by the early acting zygotic influences, up to a stage which varies with the individual case as controlled by the so-called accidents of chorionic fusion and vascular anastomosis, and then modified in the male direction by the male hormone, secreted by her twin brother and passed to her in the natural blood transfusion, causing a retracing of the path of female development, followed by development in the male direction.

The Saccus Vaginalis, Round Ligament and Gubernaculum.

For purposes of description, the term Saccus vaginalis will be used to designate the cavity or sac that develops from the vaginal process of the peritoneum in the inguinal region, in both males and females, and inguinal canal its part in the region that later closes in adult male. The term Canal of Nuck will not be used. Gubernaculum will signify the ligament of attachment of the genital cord to the bottom and wall of the saccus vaginalis if that ligament thickens and

lengthens in the abdominal cavity, and does not penetrate the inguinal region above Poupart's ligament or its equivalent. In free-martins the two conditions may apply on the different sides.

Up to 2.8 cm. the saccus and ligament are practically identical in the two sexes. In the case of the female the saccus vaginalis usually does not enlarge relatively after this stage, and though there appears to be some variation in this respect, it never approaches the male condition, in which this sac grows out through the inguinal canal to sufficient length finally to reach the bottom of the scrotum. Neither does the wall of this sac thicken in the female to the same extent as in the male. In the female the ligament lengthens and thickens in the abdomen, then shortens, while in the male, it does so in the saccus vaginalis, and to a greater extent, particularly in stages above 20.0 cm.

At 3.0 cm. (Figures 4 and 13), the female has a shallower saccus vaginalis than the male and a slightly shorter ligament. By the 5.1 cm. stage (Figures 6 and 15) the female has less than half the depth of saccus found in the male, though the wall is not noticeably thinner. The ligament is longer in the abdomen, and leads more laterally than in the male. This is shown in gross dissection better than in sections or reconstruction figures, as only dorso-ventral displacements can be shown. In both sexes, the ligament grows down inside the saccus, attached along one side by a mesentery (really retro-peritoneal at first), and to the bottom.

At 7.5 cm. (Fig. 7) in females, the ligament has elongated, broadened, and thickened in the abdomen. The saccus cavity is no deeper, though the wall with its solid bottom extends a little farther into the tissue of the inguinal region. The direction of growth of the saccus wall is more postero-lateral than ventral. In males (Fig. 16) the gubernaculum is narrower in the abdomen than in the female, but the saccus cavity is about two and one-half times as deep,

and the wall a little thicker. Its direction after entering the body wall is more ventrad.

At 9.0 cm. (Fig. 8), the round ligament is thickening in the abdomen of the female more than in the 10.0 cm. male (Fig. 17). Its direction is caudad more than ventrad in the body wall. The saccus depth has not increased materially, and the ligament thickens inside it at the same rate as in the abdomen. In the 10.0 cm. male the ligament is thinner than in the female, and tighter stretched and straighter. The saccus is incomplete in the preparation, but is very much longer and deeper than in the female even though incomplete. The gubernaculum extends down its wall, but the direction is more ventrad than in the female.

In the 15.0 cm. female (Fig. 9), a marked change is evident. The round ligament in the abdomen is much broader and thicker than in earlier stages, and the cavity of the saccus is relatively and probably actually shorter than in the 9.0 cm. stage, but its wall is thicker and its direction is still lateral, and caudal, not ventral. The genital cord has taken a decided ventral loop in the region just anterior to the attachment of the round ligament. The widening of the pelvis, rapid growth of the uterine horns, anterior to the attachment of the ligament, the retreat of the gonad, and the failure of the saccus vaginalis to penetrate the inguinal canal, have contributed to keep the growth of the ligament all in the abdomen. In the 15.0 cm. male (not figured) the gubernaculum extends almost directly from the much reduced genital cord to the body wall as a thinner flap than in the female. It is included rolled up in the inguinal canal, and instead of becoming solid, as in the female, the saccus extends ventrally and caudally as a long narrow pocket with the gubernaculum folded inside it and attached to its dorsal wall by a mesentery. The ventral margin of the gubernaculum is thickened, apparently paralleling the thickening

of the round ligament in the female. The saccus cavity was over ten times as deep as that of the female of the same size.

In the 16.8 cm. female (Fig. 10), the round ligament is thickened in the abdomen. The saccus is about one-third as deep as in the 17.5 cm. male (Fig. 18), but in it there is no thickening corresponding to the gubernaculum, nor is the saccus wall thickened as much. The direction of growth is more laterad and caudad than in the male, notwithstanding the appearance in the illustration which is somewhat distorted by dissection. The unusual depth of the saccus cavity in this female shows that there is considerable variation in the normal female, but the range in the free-martin far oversteps these bounds.

In 17.0 cm. males (Fig. 18) the saccus has a large zone of growth in walls and bottom. The gubernaculum is even more thickened in the saccus than the round ligament of the female is in the body cavity, and it is also somewhat thicker in the body cavity than in earlier male stage, causing a loop of the genital cord. The bottom of the saccus approaches the scrotum ventrally.

The 20.0 cm. female (Fig. 11) has her round ligament very thick in the abdomen, forming a thick fold between the dorsal and ventral limbs of the loop of the uterine horns on each side, with a slit-like cavity in it, the origin of which it was impossible to determine. It is not open to the coelom as is the saccus, but seems to develop by delamination in situ. The cavity of the saccus vaginalis has disappeared, and the round ligament joins the broad ligament laterally, forming a thin flap of mesentery along the body wall for some distance, but with no great thickening in the body wall where it enters. It just spreads out into the surrounding tissues and disappears.

In the male at 20.0 cm. (Fig. 19), the gubernaculum extends slightly ventrally from the small genital cord to the saccus opening in the body wall. It is thicker and relatively shorter than in earlier stages. In the saccus it is thickening and less folded. The saccus extends deeper ventrally toward the scrotum, almost reaching

its top.

From the above details it appears that the gubernaculum thickens less than the round ligament at this stage. Its growth down the saccus is great, while usually no appreciable saccus appears in the female, and for this reason the great thickening in the female occurs all in the body cavity, while that in the male in in the saccus. The increase in length in the female, and the attachment to the uterine horns nearer their union to form the corpus uteri, causes a loop in the uterine horns, in addition to the swelling and loop of the round ligament between them. This ligament seems to shorten and thin out in later stages in the female, in the same manner as the gubernaculum shortens in drawing the testis into the inguinal canal and down to the scrotal sac. In any case, it does not retain through later stages its relative size as compared to the cornua uteri.

In Free-martins:

In 9.0 cm. free-martin No. 1 (Fig. 20), the ligament is not thickened in the abdomen as in the female, is relatively a little longer than in the male, but bends slightly ventrad as in the female. The genital cord also takes the ventral bend characteristic of the female, apparently due to the lengthening of the cord, not to a pull by the ligament. The saccus vaginalis is deeper than in the female of the same size, but its walls no thicker, and its direction caudad as in the female. While this specimen is intermediate in condition between the sexes in the round ligament, it is still predominantly female.

The 9.0 cm. free-martin No. 2 (Fig. 21) has the gubernaculum slightly thicker in the abdomen than in the normal male, and longer, and less direct from the cord to the saccus opening. The direction of the saccus is ventrad, somewhat as in the male, and the bottom of the saccus is denser, thicker, and more actively growing than in the female. The ventral loop of the genital cords of the female is wanting here. This free-martin is more decidedly male in condition than No.1

and has a gubernaculum instead of a round ligament.

The 11.75 cm. free-martin (Fig. 22) has its round ligament thickened and curved in the abdomen, somewhat as in the 9.3 cm. female, but the curve is dorsad not ventrad as in the female. The saccus vaginalis is at least ten times as long as in the 15.0 cm. female (Fig. 9), and three times as long as in the 9.0 cm. female. The ligament extends to the bottom of this sac as in the male, but is not yet thickened in the sac, nor is the sac wall much thickened. The direction of growth is ventrad as in the male. This free-martin has male and female characters somewhat evenly balanced. It suggests a fairly advanced female condition reversed by the action of the male hormone, taking effect late, by reason of late chorionic vascular anastomosis.

In the 17.0 cm. free-martin (Fig. 23) the gubernaculum is relatively long in the abdomen, but only slightly thickened there, and has a double curve instead of being tight stretched. The saccus is much longer than appears in the figure reaching almost to the udder primordium, and the gubernaculum is much thickened in the saccus as in the male. The walls of the saccus are thick and the direction of the active growth is ventrad. This is decidedly male in type of development, but has evidences of female early stages.

The 20.0 cm. free-martin (Fig. 24) has a very marked thickening and broadening of the gubernaculum in the abdomen, but less than in the female, and farther laterad, toward the body wall. It forms a decided bulb in the body cavity lateral to the umbilical arteries, but extends medially to its attachment to the genital cord, as a thin sheet. The saccus invades the body wall ventrally and caudally, about one-third as far as in the male of this size, while in the female none at all is evident. Here the gubernaculum thickens in the saccus and this thickening is continuous with that in the abdomen.

The saccus bottom is growing nearer the udder region, but no scrotum appears.

That this structure is a female one being modified in the male direction is shown by the tendency to thicken in the body cavity, but also inside a long saccus vaginalis.

An interesting case in this connection is that of a 23.0 cm. free-martin, in which the left side has a testis already drawn into the saccus, and a gubernaculum thickened only in the sac, while the right side, on the contrary, has a round ligament almost all thickened in the abdomen pushing the gonad farther back from the saccus opening. This seems to be male on one side and female on the other, if one does not examine too closely. But on closer examination, the gonads are seen to be alike except in position. The anomaly is due to the failure of the right sac to penetrate the inguinal canal, and develop, while the left did so, and so one side developed in the female manner and the other in the male. This can be understood when it is considered how far the left side, as a rule, is in advance of the right in development, particularly of the male characters. The stimulation of the vaginal sac caused it to reach the inguinal canal on that side before it closed, or caught the left primordium at its critical stage and caused the right to grow too slowly to pass the closing canal, or missed its critical stage. In many free-martins the sacs fail to penetrate on both sides as shown in Lillie's ('17) paper and in many they penetrate on both. There would appear to be a time when the penetration by the saccus is inhibited, if the saccus has not already passed a certain stage or point in the inguinal canal. The left seems to pass this stage earlier than the right and to be in a condition to penetrate more often than the right.

The Fate of the Mesonephros

In the female:

The mesonephros reaches its maximum size in embryos between 5.1 and 7.5 cm. For at 7.5 cm. it is shorter than at 5.1 cm. Bergschicker ('12) says the maximum isrreached at approximately 6.5 cm. neck-rump length. Resorption appears to be greatest in an anteri-posterior direction, but some seems to go on at the posterior end. The retreat of the gonad may add to the appearance of posterior degeneration. Bergschicker ('12) shows there are fluctuations in both sexes, and this study did not make comparisons of animals of the same age and sex.

By 9.0 cm. (Fig. 8) in the female, the mesonephros shows considerable decrease in size, and this takes place principally by the degeneration of the cranial group of tubules and glomeruli.

At 15.0 cm. (Fig. 9) the female shows a great reduction in the number of glomeruli leaving a mass of tubules and a few glomeruli. The rete ovarii still passes out into the mesonephros and leads to a glomerulus, but no evident tubular connection either to the glomerulus or to the Wolffian duct is present. As was noted under the Müllerian duct development, the rete leads on to the ostium tubae abdominale, but its significance was not clear from this study.

At 16.5 cm. (Fig. 10) in the female, the cranial group of tubules are not retained in any capacity and the whole mesonephros has undergone further degeneration.

At 20.0 cm. (Fig. 11) the rete ovarii extends anteriorly from the gonad and reaches the coelomic surface, forming a few tubules opening into the body cavity, and covered with columnar epithelium on their coelomic edges. This joins the ostium anteriorly, but has no connection with mesonephric tubules or duct, and fades out posteriorly. No glomeruli and only a few small tubule remnants are present in the mesonephros itself, forming an epoophoron, as the tubules connected to the rete are

interpreted as remnants of the anterior group, and a paroophoron, consisting of a few tubules at the posterior end of the gonad.

In males:-

The condition parallels that in females up to 3.5 cm. (Fig. 14) where the mesonephros appears larger in diameter, but not longer, than in the female. At 5.1 cm. (Fig. 15) it is longer and the cranial group of tubules has not degenerated as much as in the female while the caudal group has. So its anterior part is larger in diameter, than in the female. This difference gets more marked at 7.5 cm. (Fig. 16) and, at 10.0 cm. (Fig. 17), a male has it less degenerated in the anterior end than a 9.0 cm. female (Fig. 8), but just as much in the caudal group. At 17.0 cm. (Fig. 18) the anterior or cranial group of tubules remains and forms part of the epididymis connecting the rete testis directly to the Wolffian duct, but the caudal group has degenerated and glomeruli are found only as rudiments in the paradidymis or organ of Giraldes and in a ductus aberrans. Ductuli efferentes are forming a connection of the Wolffian duct with the rete tubules of the testis, but the glomeruli of these cranial mesonephric tubules are gone. At 20.0 cm. (Fig. 19) this connection of the ductuli efferentes from Wolffian duct to rete testis is plain and easily traced. Such connection was lost in the female, where the rete led out to the ostium at this stage. The caudal group of mesonephric tubules has formed a paradidymis, and a ductus aberrans extends off by itself in the mesentery with three small enlargements on its tubule, like degenerate glomeruli. The whole mesonephros is slower to degenerate in the male than in the female, especially in the later stages.

In the free-martin:-

In the 9.3 cm. free-martin No. 1 (Fig. 20) the mesonephros is larger than in the female of the same size. The caudal group of tubules are degenerating a little more slowly than in the female,

but the cranial group retain their connection to the rete and Wolffian duct as in males. Glomeruli are present in both groups of tubules as in the normals of both sexes. This free-martin resembles the male in longer mesonephros and connection of the cranial tubules to the rete of the gonad. It resembles the female in having the rete linked to the ostium, but less definitely so than in the female. The rete is not yet organized into definite tubules.

The 9.0 cm. free-martin No. 2 (Fig. 21) has more reduced cranial tubules but a total length greater than in females. The caudal group of tubules is not so shrunken as in No. 1. The connection of rete and coelom is smaller than in the female, but the rete is connected to the Wolffian duct by the cranial group of tubules and glomeruli are present.

The 11.75 cm. free-martin (Fig. 22) has a marked constriction of the mesonephros between the cranial and caudal groups of tubules. In one place there is but a single tubule in cross-section between them. The cranial tubules persist without any connection to a Wolffian duct, which is wanting in this region of this free-martin. The ductuli efferentes are present and the rete is connected to the hydatid of Morgagni or ostium remnant, and only to the posterior few tubules of the cranial group. Though no Wolffian duct could be distinguished from the tubules, it probably exists coiled upamong them. Posterior to the gonad, tubules are more numerous and a few degenerating glomeruli are to be found, but no Wolffian duct. The Wolffian body as a whole is almost as small as that of a 13.0 cm. female, but degenerates in the male manner, after losing its ducts as much as a 15.0 cm. female (Fig. 9).

The 17.0 cm. (Fig. 23) free-martin has a much shortened mesonephros in which the cranial group of tubules seems to be involved in the retreat of the gonad, for its epididymis and the caudal group appear dorsal to the cranial ones. The rete has lost its connection to the hydatid, but retains it to the cranialgroup of tubules and thus to the Wolffian duct. Glomeruli are absent. There is more

mesonephros retained than in either male or female, but the relative position and connections of parts retained are male, with slight modification.

In the 20.0 cm. free-martin (Fig. 24) the rete retains its connection to the anterior tubule remains in the epididymis, but has lost it with the hydatid which is also absent. A ductus aberrans is represented by a single isolated tubule, extending forward from the mesonephros in the body wall, separate from the other tubules. Glomeruli are absent. While the cranial group of tubules is not yet quite as much stimulated as in the male, where they form the ductuli efferentes of the epididymis, there is more mesonephros as a whole than in the male or female.

The degeneration of the mesonephros in the free-martin is slower than in either sex normal, but in general, it follows the male method of disappearance, in that the cranial group of tubules form ductuli efferentes of the epididymis.

The Urogenital Sinus and Its Derivatives.

As before described, the separation of the urogenital sinus from the rectum is complete by th 2.4 cm. stage. In the female the perineum remains short, and the urogenital sinus opens to the exterior through the urogenital plate at this early stage, while in the male the sinus remains closed, and a ridge or raphe forms ventrally over the urogenital sinus, which is prolonged to the tip of the phallus by a narrow vertical plate of cells derived from the urogenital plate. The penis migrates ventrally along the belly wall to its definitive position close to the umbilicus. This greatly elongates the urethra in the male, particularly in the pars membranacea and pars cavernosa, and directs it antero-ventrally, instead of posteriorly as in the female.

In females at 2.8 cm. (Fig. 3) the urogenital plate, which extends from the perineum to the tip of the phallus, is perforated by the posterior continuation of the urogenital sinus lumen in the form of a narrow slit to the exterior, extending some distance ventrally from the perineum, but leaving the ventral two-thirds of the plate filled with the epithelium of its walls. This plate extends along the dorsal surface of the phallus to its tip. The primordia of the bulbo-vestibular glands are already present, as small lateral out-pouchings of the lining epithelium of the two dorsal-grooves in the side walls of the sinus. While only about 100 micra long, they turn forward as distinct glands, but are not so far dorsad as in the male of this size, and are slightly more apparent. From these glands forward, the sinus, flattened out laterally, bends slightly ventrad, and a ridge develops in its dorsal wall, extending farther into the lumen as one proceeds forward, till the sinus itself becomes a narrow crescent in section outline where the Wolffian ducts open into it. This they do by penetrating the inner walls of this crescent from the interior of the ridge. The Müllerian ducts do not reach the sinus at this stage, but they do at 3.0 cm. (Fig. 4), just posterior to the Wolffian ducts. The distance on the urethra proper from ureters to Wolffian ducts is longer in the 3.0 cm. stage.

At 3.8 cm. (Fig. 5), the sinus is a little longer, the bulbo-vestibular glands larger, and the sex ducts open into a sinus, oval in cross-section. The urogenital plate still extends to the tip of the phallus. The posterior vulva opening has enlarged in all dimensions, and is wide open for almost the entire depth of the plate. The distance on the urethra, from the ureters to the Wolffian ducts, is longer, the perineum is a little wider, and the phallus, a little more recurved, than in younger stages. The little regions of growth behind the vagina are now incorporated into it.

At 5.1 cm. the urogenital plate extends forward into the tip of

the phallus as a pouch, filled with epithelium, and roofed over by the recurved tip of the phallus, which flattens out at the 16.5 cm. stage. The perineum widens a little by the 5.1 cm. stage, (Fig. 8), but thereafter it just keeps pace with the growth of the surrounding parts and gets relatively narrower from the 16.5 cm. stage on. The vulva enlarges somewhat, but, while at 5.1 cm. it enters in an antero-dorsal direction, its floor gradually flattens out and by the 15.0 cm. stage (Fig. 9), it leads nearly horizontally into the vestibule, while at 23.0 cm. it is horizontal (Fig. 11). The vulva keeps pace with the growth of the surrounding structures.

The bulbo-vestibular glands grow out laterally from the sides of the sinus where the vulva leads into the vestibule, and they begin to branch at the 7.5 cm. stage and thereafter become more branched and much larger, till at 15.0 cm. they penetrate beyond the muscular coat of the vestibule, enclosed in a muscular coat of their own and covered by the primordium of the constrictor vulvae muscle. They continue to increase relatively to the 23.0 cm. stage (Fig. 11). Their ducts open to the vestibule farther ventrally on the sides of the vestibule than do their homologues in the male.

The vestibule becomes relatively shorter and larger in lumen, and becomes almost double by reason of two ridges, one in each lateral wall, that almost separate a longitudinally wrinkled dorsal corridor, which in later stages leads straight forward into the vagina as in 15.0 cm. female (Fig. 9) from a ventral corridor, leading forward to the urethra proper.

At 5.1 cm. the vagina and Wolffian ducts open into the sinus, one behind the other, from the walls of a ridge in the dorsal sinus wall, round a tongue of tissue as described under Müllerian ducts, and the vagina has two backward projecting pouches at its posterior end. By 7.0 cm. (Fig. 8) the Wolffian ducts join the vagina before it,

to the vestibule, and at the 16.0 cm. stage the vagina opens straight anteriorly from the dorsal corridor of the vestibule as in later stages.

So-called homologues of the prostates, the para-urethral ducts do not make their appearance before the 20.0 cm. stage (Fig. 11), when they appear as two or three small side ducts off the dorsolateral grooves of the vestibule, a short distance posterior to the vagina orifice. They do not develop in the postero-anterior direction characteristic of the male and they are much belated in appearance.

Anterior to the vagino-vestibular opening, the urethra proper extends forward to the ureter openings. As described before, this distance lengthens rapidly in the female to become double that in the male by the 9.0 cm. stage, (Fig. 8) and still longer relatively, later. At 5.0 cm. it passes forward without narrowing noticeably, changes from a square cross-section outline to a crescentic one, and develops a ridge in its dorsal wall, through which the ureters enter it. In later stages, when the suburethral diverticulum develops from 9.0 cm. upwards (Fig. 8), it has a sudden decrease in lumen, just in front of the vagina opening, with the suburethral diverticulum developing as a forward projecting blind pouch, ventral to its narrow posterior end. This diverticulum increases in size, and finally, by the 15.0 cm. stage (Fig. 9), it forms an anterior continuation of the ventral corridor of the vestibule, with the very narrow typically female urethra, opening into its dorsal wall near its anterior end. This diverticulum enlarges and lengthens up to the 20.0 cm. stage (Fig. 11). This seems to have no homologue in the male.

In males:-

The urogenital plate does not become perforated as in the female, and, at 2.8 cm. (Fig. 12), its edges in the middle and dorsal part have begun to grow together to form a raphe ventrally from the perineum.

In the dorsal surface of the phallus, its margins next the skin are closer approximated than in the female. The whole of it is filled with epithelium and the perineum is but slightly broader dorso-ventrally than in the female. Bulbo-urethral gland primordia are present as homologues of the bulbo-vestibular glands, but more dorsally placed. The lumen of the sinus anterior to these glands is narrower than in the female. The Wolffian ducts enter at its anterior end, but the ridge found in the female is wanting. The Müllerian ducts have not yet nearly reached the sinus. In the specimen studied there was a small blind pouch, lined with Wolffian duct epithelium, caudal to the right Wolffian duct, in the dorsal sinus wall, but as it does not appear in later stages it is taken to be of no sexual significance. The ureters join the sides of the Wolffian ducts and sinus at their point of junction, and the sinus extends forward as the urethra or neck of the allantois, without narrowing. By 3.0 cm. (Fig. 13), the urogenital sinus reaches the surface only on the dorsal surface of the phallus, and is filled with epithelium throughout. Bulbo-urethral glands are more developed than their homologues in the female. The sinus, anterior to these ducts, has a larger dorsal ridge than in the female, and from it the Müllerian and Wolffian ducts join the sinus together, but the Müllerians extend farther back as two diverticula. The urethra proper is shorter than in the female, but broader. The 3.4 cm. male (Fig. 34), has the following changes;- the migration of the penis forward along the ventral body wall and the widening of the perineum has continued, the bulbo-urethral gland apertures have moved ventrally on the sides of the sinus and the sinus dorsal wall has a pocket, the supra-urethral diverticulum, extending dorsally toward the rectum. The length of the prostatic urethra is longer than in the female, and the Müllerian ducts join the Wolffian ducts, and the latter join the sinus, but the ridge in which they lie is relatively smaller than in earlier stages. The sex differences at this stage are;- the recurved unperforated

cavernous urethra, the migrated penis, the dorsal pouch in the bulbo-urethral region, the more ventral bulbo-urethral glands, the longer prostatic urethra, the short wider urethra proper, and the wider perineum of the male, as compared with the female.

At 5.1 cm. (Fig. 15) the migration of the penis past the scrotum has lengthened the cavernous urethra, which is narrower and perforated some distance from the prostatic part toward the penis. The corpus cavernosum urethrae is developing along its antero-dorsal surface, and surrounds all but its ventral surface. The bulbo-urethral glands under the primordium of the bulbo-cavernous muscle and the supra-urethral diverticulum at the bend, are longer than in earlier stages. The prostatic urethra has narrowed very much just anterior to the ducts of the bulbo-urethral glands and prostates first appear as small solid diverticula off the dorsal and lateral grooves of the sinus, called the prostatic grooves. They appear first at the posterior end of the prostatic urethra and spread anteriorly and circumferentially in later stages. The prostatic urethra is relatively longer and narrower than in the female. The Müllerian ducts open into the sinus separately posterior to the Wolffian ducts, and the urethra proper is shorter than in the female.

By the 7.3 cm. stage (Fig. 16) the urogenital plate remnant has its definitive position at the under surface of the gland penis, and perforation of the cavernous urethra is almost complete. The sigmoid flexure of the penis is beginning to develop and the urethra traverses it. The supra-urethral diverticulum at the bend is smaller relatively. Prostates surround the prostatic urethra and extend forward almost to the vagina opening, and the bulbo-urethrals begin to branch. The urethra proper is short, and the prostatic urethra is about the same length in males and females at this stage.

At 10.0 cm. (Fig. 17), the prepuce begins to develop round the glans, and the whole penis points more anteriorly than in earlier stages. The dorsal diverticulum is larger and the bulbo-urethrals longer and more branched. Prostates are more profuse and extend more around the elongated prostatic urethra, which is narrower than in the

female. Vagina masculina and Wolffian ducts join it separately, and the prostates reach the vagina opening. No suburethral diverticulum develops in the male, but the urethra narrows anterior to the vagina.

At 17.3 cm. (Fig. 18), the sigmoid flexure of the penis is well developed and the cavernous urethra long. The greatly enlarged bulbo-urethral glands open into the dorsal diverticulum at the bend of the urethra. The prostatic urethra is three times as long as in the female and narrower, and the prostates surround it and the vagina. The urethra proper is not narrowed as in the female, and is only half as long. The vagina does not open to the sinus, but is blind, both anteriorly and posteriorly.

The perforation of the urethra reaches the surface in the 20.3 cm. male (Fig. 19). All the male characters are more advanced except the supra-urethral diverticulum at the bend, which is smaller. Prostates are taller dorsally, but few, small, and scattered, ventrally.

In free-martins:-

The 9.3 cm. free-martin No. 1 (Fig. 20) has a set of female urogenital sinus derivatives which correspond to those of a normal female of somewhat younger stage. The floor of the vulva and vestibule is flatter and the vestibule is short, but not quite so large in diameter, as in a female of this size. The perineum is as in the female. The bulbo-vestibular glands are not so far advanced. The Wolffian ducts join the vagina, then both join the sinus, as in the female. The urethra narrows in front of the suburethral diverticulum and is even longer than in the female of this size. No prostates are found. There is no resemblance to the sinus derivatives of the male in any particular.

In 9.8 cm. free-martin No. 2 (Fig. 21), the urogenital sinus derivatives are also female in every respect, but of slightly reduced size. It even has the same small pocket of epithelium under the tip of the glans clitoridis, as well as the female vulva opening. The

vestibule is short, and wide in diameter; bulbo-vestibular glands are female in type, but less branched and smaller, than in No. 1. However, the vagina and Wolffian ducts open to the vestibule separately like a male. The urethra proper is twice as long as in the 10.0 cm. male (Fig. 17). The suburethral diverticulum is not developed, but the urethra narrows after the female fashion. Perineum is female in type.

The above details show that, of the two 9.0 cm. free-martins studied No. 1 is more advanced in female characters than No. 2, in respect to ureters, Wolffian duct connections, and vagina, all mesodermal derivatives, but neither is in any respect male in the derivatives of the urogenital sinus, which is derived from entoderm.

The 11.75 cm. free-martin has a clitoris reduced in size and a female type of perineum, vulva, and vestibule. (Fig. 27) Bulbo-vestibular glands are more advanced than in the 9.0 cm. free-martins or normal females of that size, and the vestibule is shorter. No prostatic homologues are found. Vagina and Wolffian ducts unite, then open into the sinus as in the female. There is no definite suburethral diverticulum, but the urethra narrows in front of a dip in the vestibule floor as in a much younger female, and a degeneration region is found dorsal to the anterior end of the ventral vestibular corridor, where the urethra would be, if the suburethral diverticulum had developed. This looks as though some degeneration of a female condition were going on. The urethra is twice as long as in a 9.0 cm. female and four times as long as in a 10.0 cm. male. This free-martin is also female in sinus derivatives, but with modifications in the male direction in the mesodermal derivatives, the junction of the urethra to the suburethral diverticulum or vestibule.

The 17.0 cm. free-martin (Fig. 37) also has a female set of urogenital sinus derivatives. The vulva is open wide, the bulbo-vestibular glands are well developed, the vestibule wide and short, the suburethral diverticulum large, Wolffian ducts join the vagina, then both join the vestibule, and the narrowed urethra is 4.1/3.4 as long as in the female

of this size while the male has it 1.8/2.4 as long as the female. The small primordium of the para-urethral ducts appears as in a female of 20.0 cm. (Fig. 11) on the dorso-lateral groove of vestibule on each side. If this is a homologue of the prostate as given by Neugebauer ('08) it develops late here as in the female, and in the female fashion, not postero-anteriorly as in the male.

The 20.0 cm. free-martin (Fig. 24) has a much more advanced female type of vulva and a flattened out vulvo-vestibular canal, while the bulbo-vestibular glands are better developed. Other advances in development are shown by the shortening and widening of the vestibule, the method of union of the vagina masculina with the Wolffian ducts and sinus, the suburethral diverticulum, the length of the urethra proper, and even in th presence and condition of the two small para-urethral ducts of Skeene as in the 20.0 cm. female, but larger. And so here, too, the female conditions are retained, but the para-urethral ducts are ahead of the female, but are evidently secondary in their time of stimulation, just as in the female, but this study does not take in late enough stages to decide their exact homology.

The sexual differences in the development of the derivatives of the urogenital sinus and plate, brought out by this study, are as follows:-

(1) The development of the long cavernous urethra with sigmoid flexure in the male, lacking in the female, is accompanied in the male by widening of the perineum and closing over of the urogenital plate, with consequent ventro-anterior migration of the penis. Its absence in the female is accompanied by greater recurving of the clitoris.

(2) Development of the small supra-urethral diverticulum in the male at the bend of the urethra and junction of the cavernous and prostatic portions, to receive the bulbo-urethral glands, with no homologue in the female.

(3) The long narrow prostatic portion of the urethra in the male as contrasted with the wide short vestibule in the female.

(4) The difference in type of the bulbo-urethral glands in males from that of the bulbo-vestibulars in the female, their homologues.

(5) The development in the male of profuse prostates, surrounding the prostatic urethra from the bulbo-urethral gland region to the openings of the Wolffian ducts, and spreading in a postero-anterior direction. They appear first at 5.1 cm. stage. The para-urethral ducts of the female, given by Neugebauer as the homologues of the prostates, do not appear in females before the 20.0 cm. stage, when two or three appear in the dorso-lateral grooves of the vestibule, near the vagina opening, not near the bulbo-vestibular glands as in males.

(6) The absence in the male of a homologue for the sub-urethral diverticulum of the female, and the failure of its urethra to narrow in front of the Wolffian duct openings as in females.

(7) The short urethra proper in the male as contrasted with one twice as long or more, in the female.

(8) The great development of the long corpus cavernosus urethrae in the male, with its sigmoid flexure, as contrasted with its almost rudimentary condition as the corpus cavernosum clitoridis of the female, short and sinuous.

(9) The opening of the Wolffian ducts separately anterior to the vagina masculina in the male, as contrasted with their union with the vagina before opening as one to the vestibule in the female.

In all the above features, except the last, the free-martin is definitely and unmistakably female as far as shown by the specimens studied. The structures mentioned in (9) above are of mesodermal origin, which may in a measure account for their being influenced in the male direction, while the rest of the sinus derivatives, except the strip between the ureters and the Wolffian ducts on the dorsal

urethral wall, are of ektodermal origin and are essentially unaffected, and remain female in mode of development.

The Phallus and External Genitalia.

In females:

The female external genitalia differ from those of the male in the perforation of the urogenital plate in early stages. This takes place before the 2.8 cm. stage. At that time the position of the phallus is approximately the same in the two sexes, and both have genital swellings and teat primordia. In the female, as time goes on, the phallus becomes more recurved toward the anal opening, the vulva more open, and the perineum no wider relatively. The genital swellings in cattle, unlike those in the human, as described by McMurrick in his "Development of the Human Body", do not take part in the formation of the labia majora, as is shown by F. R. Lillie in his unpublished work on the homologies and fate of the external genitalia in cattle, but completely disappear in females at about 10.0 cm. - 12.0 cm., and labia majora are absent in cattle. The lips of the genital plate form the genital folds and, finally, the labia minora. The place of the absent labia majora is taken, in later stages, by the labia minora or nymphae, which grow out on the sides of the recurved phallus and approach its tip, finally overlapping it in the older stages, though not by the 20.0 cm. stage. The tip of the phallus is cavernous and forms the glans clitoridis. In early stages the glans has an undercut groove in its dorsal part, filled with stratified epithelium. This disappears in later stages. The thick ventral wall of the vulva becomes cavernous, as the corpus cavernosum clitoridis, which is sinuous and does not have the great increase in lenght so characteristic of the male. The glans clitoridis is knob-shaped at first, but by the 20.0 cm. stage it is pointed. The udder and teats develop and the genital swellings give rise to

small scrotal prominences which disappear after the 10.0 cm. stage.

In males:

The phallus migrates along the ventral body wall, following failure of the urogenital plate to perforate, as it does in females: the perineum grows ventrally over the dorsal part of the plate; the lips of the plate fuse to produce a raphe on the body wall. Thus the phallus is pushed forward to the umbilicus region, and comes finally to point antero-ventrally. The sigmoid flexure of the much elongated penis, including the urethra and corpus cavernosum, develops from before the 7.5 cm. stage (Fig. 16). The genital swellings develop into a scrotum, no udder develops, and teats remain rudimentary. The sides of the phallus develop into a sheath of skin as a prepuce at about 10.0 cm. (Fig. 17), and a fossa develops round the glans penis, which glans remains projecting forward as the end of the corpus cavernosum urethrae. The glans remains open ventrally allowing the urethra to open on its ventral face, and is attached to the prepuce by a frenulum or strip which takes a spiral course along one side of the glans, from a ventral position near the tip, to one slightly to the left at the bottom of the fossa.

In the free-martin:

The phallus development of the free-martin is completely and exclusively of the female type, so far as seen in the 38 free-martins collected while pursuing this study, and Lillie ('17,'23) does not record any modification of the external genitalia in the male direction. Keller and Tandler ('16) mention finding some such modifications of the external genitalia, but do not describe them. The only cases properly described are that of Numan ('43,'44), and he had only the dealer's word that it was twin to a bull. [illegible] Such cases occur in single born calves, and therefore need not be due to the situation during intra-uterine life, but may very well be zygotic intersexes, of which one would expect to find the same percentage among free-martins as among single born heifers,

and of which there are some recorded cases. There is some reduction in size of the external genitalia in some cases, e. g. the 9.0 cm. free-martin # 5. But there is no sign in any of these free-martins of forward migration of the phallus, nor of closure of the vulva by overgrowth of the perineum as in the male. These minor modifications found are all to be found in normal females so far as noted. In free-martins the scrotal prominences disappear as in the normal female, and the udder forms, but still remains small, even when correlated in the individual with descent of the gonads as in males.

Summary and Discussion.

(1) The gonad of the free-martin develops as an ovary to a varying degree of differentiation, depending on the time of initial reaction to the male hormone, and may go so far in the female direction of its differentiation as to develop a considerable layer of secondary sex-cords, which, under the influence of the male hormone, undergo degeneration, and apparently entirely disappear. The sex-cord region, in all cases examined, is cut off from the epithelium covering the gonad by a tunica albuginea resembling that of a male of smaller size than the free-martin, and the rete of the gonad assumes a position in the gonad correlated with the degree of development in the male direction reached by the other sex organs.

(2) A sexual difference is evident in the much longer distance on the urethra between the openings of the ureters and those of the Wolffian ducts in the female as compared with the male. These structures have a common origin in both males and females, but the ureters migrate anteriorly along the urethra in both sexes. The free-martin is usually intermediate in length of this tube, but is sometimes even longer than the corresponding female. It does not approach the male condition in the cases studied.

(3) In females progressive narrowing and degeneration of the Wolffian ducts set in at about 5.1 cm. stage and continues, at a rate varying in different individuals, till, at 15.0 cm., the ducts are intermittent anterior to the vagina, and, at 28.0 cm. they are present only in the uterus wall, as solid stripe, and as minute isolated segments, in the wall of the anterior half of the vagina, but continuous in the posterior half, and with a lumen in the posterior one-third of the vagina wall. They have lost all connection to the rete of the gonad and to the cranial group of mesonephric tubules.

In males these ducts continue to develop and have a lumen throughout. They may even occupy the whole of the genital cord in places where the Müllerian ducts are degenerated completely. They retain a connection through the cranial group of mesonephric tubules with the rete testis, in the epididymis.

In free-martins the condition of the ducts varies. In one case (11.75 cm.) they have degenerated in the anterior part as in the female, and have lost all connection through the cranial group of mesonephric tubules to the rete. In the other cases studied, they retain this connection and are male in condition, but are comparable to males of younger stages. In all, they appear to have behaved as in the female for a considerable time, and to have degenerated somewhat, and then been stimulated again.

(4) In females the Wolffian ducts in later stages join the vagina before opening into the sinus, while in males they open separately throughout the series as in early stages of both sexes. In free-martins the condition is variable. Sometimes it is as in females, and at other times as in males. This bears a relation to the degree of maleness of the other organs in each individual.

(5) The seminal vesicle primordia begin to show development in the 7.5 cm. females, and five, or possibly more, begin development in the stages studied. But none reach a size appreciable in

gross dissection, and in later stages they appear on an interrupted Wolffian duct, and, in many cases, on isolated parts of it, and always farther forward than in males of like size. In the last stage studied they are found in the lateral walls of the cervix uteri and anterior part of the vagina. The number of minute vesicles is a sex characteristic.

In males usually one, or at most two, occur, of which the posterior one does not develop, while the anterior one on each side grows strongly to a considerable length before branching and the opening of its single duct in later stages is progressively nearer the posterior end of the Wolffian duct, into which it opens. The large size and profuse branching of the single seminal vesicle on each side, in the male, is a striking characteristic of the sex.

In free-martins the condition as regards seminal vesicles is varied. All appear to have developed as females with some inhibition of the vesicle growth, followed by increased stimulation, which causes increased size, like that in the male, but with increased number as in the female, or even more, e. g., the 20,3 cm. free-martin with 11 diverticula on each side. This is again a case of a male type of development, imposed upon a set of female primordia, the degree of which appears to be a function of the time of inception of the male hormone, and the time elapsed from inception to the stage studied.

(6) There appears to be a sexual difference in the rate of growth of the Müllerian ducts in stages that appear to be sexually indifferent externally. In females the Müllerian ducts reach the sinus earlier than in males, and as this occurs in the female, at least, before either sex has interstitial cells, it is believed to be zygotically controlled.

(7) In females the Müllerian ducts continue to develop into the ostium, fallopian tubes, cornua uteri, corpus uteri, cervix uteri, and vagina, and remain continuous and patent throughout. The retreat of the gonad and the altered proportions of the pelvic

region cause a looping of the cornua uteri and tubes. The vagina and the uterus increase greatly in diameter in later stages, and the former opens straight posteriorly into the dorsal corridor of the vestibule.

In males the ostium degenerates and leaves only the hydatid of Morgagni, which is not always present, even up to 20.0 cm. stages, and the duct itself degenerates completely in the tubar and cornua uteri regions. In all the stages studied, a remnant of the corpus uteri is found as a vesicle, blind both anteriorly and posteriorly, at the junction of the genital cords, or as a solid strip in the region where corpus and cervix uteri would be in a female. The vagina masculina is present in stages studied, but has degenerated to a vesicle filled with epithelium, dorsal to the openings of the Wolffian ducts into the sinus, by the 20.0 cm. stage.

In free-martins degeneration of the Müllerian ducts goes on as in the male, but it has started later, after the duct had attained a more advanced state than in the normal male, and, while degeneration seems to have been proceeding more or less uniformly throughout the duct length, it disappears first near the anterior end, in the tubar region, and last, where the uterus and vagina would be. Here again the two 9.0 cm. free-martins show a variation in the condition of these ducts, correlated with that of the gonad and Wolffian ducts, which points to a difference in time of onset of the male hormone, and consequent longer time of degeneration in No. 2, acting on a less advanced stage of female differentiation, as compared with No. 1, with later onset and shorter duration, acting on a much more advanced stage of female development. In the oldest free-martin no hydatid is found as in some males. The other three present a series in degeneration of the duct derivatives as in males, except that the onset of degeneration has occurred at various stages, and therefore has reached various stages, always comparable to those of younger males.

(8) In both sexes, and in the free-martin, the state of de-

velopment of both sets of ducts on the left side is more advanced than those on the right side, and this is particularly true of develppment in the male direction. The retreat of the gonad and of the mesonephros and the loop of the genital cords all give evidence of this.

(3) In females, the saccus vaginalis usually fails to develop to any considerable depth, while, in the male, it grows ventro-laterally through the inguinal canal to the top of the scrotum, in the specimens studied. The derivatives of the inguinal ligament, the round ligament of the female, and the gubernaculum testis, in the male, while they behave in much the same manner, are made to appear to do differently, by the differing development of the vaginal process derivatives, in the two sexes. Both ligaments elongate, then thicken for a time, then shorten; and, in the stages beyond this study, become reduced to their definitive condition, the gubernaculum in the bottom of the scrotum, and the round ligament in the body cavity. In the stages covered by this study, the round ligament lengthens and thickens in the abdomen, because there is no saccus to receive it, and the gubernaculum lengthens, partly in the abdomen, but mostly in the saccus vaginalis, and thickens wholly in the saccus. Part of the round ligament is taken up into the walls of the cornua uteri also, but it thickens slightly more than the gubernaculum in the stages studied.

Free-martins are intermediate. The same difference in degree of maleness is found in the two free-martins of the same age (9.0 cm.). The others give a series of stages in which the saccus is deeper than in females, but not so deep as in males of the same age, and the thickening of the inguinal ligament derivative occurs, partly in the saccus, and partly in the abdomen, depending on the depth of the saccus. In the two latest stages, studied for this paper, the saccus had penetrated the inguinal canal on both sides, but in some cases it penetrates on one side only, and that side develops as in a male, while the other side develops as in a female. There would appear to be a time after

which the canal cannot be penetrated, and the left side saccus sometimes is far enough in advance of the right one to penetrate, while the right does not. In that case the left side develops a typical gubernaculum, the right, a typical round ligament.

(10) There is a sexual difference in the method and degree of degeneration of the mesonephros. In females it takes place much more uniformly throughout its entire length, and the cranial group of tubules do not retain a connection with the rete of the gonad and the Wolffian duct, as is the case with males. But the rete of the ovary retains some connection through the degenerated mesonephros to the ostium. An epoophoron, from the cranial group of tubules, and a paroophoron, of a few tubules at the posterior end of the gonad, from the caudal group of tubules, remain at the 20.0 cm. stage.

In males, the cranial group of tubules retain their connection with both rete testis and Wolffian duct as part of the epididymis, and do not degenerate as in females. The caudal group of tubules degenerates as in the female, but remnants form the paradidymis, and a single isolated one, the vas or ductus aberrans. The whole mesonephros is slightly slower to degenerate in the male, and the rete testis does not connect to the ostium. Glomeruli disappear after 17.5 cm. stage, and before 20.0 cm. stage in both sexes.

In free-martins, in all those studied, except the 11.75 cm. one, where the Wolffian ducts are absent in the anterior region, the cranial group of tubules act as a connection of the Wolffian duct to the rete of the gonad as in males, and, even in that case, with no anterior end of the Wolffian duct, the cranial group of tubules is retained. In the later stages it forms part of the epididymis as in males, but is behind the normal male, in state of development. In early stages studied, the rete is connected to the ostium remnant, though not so well as in females, while in later stages it is not, and resembles a younger male condition. The characteristic paradidymis

and vas aberrans, of the male, are found in the later stages, with more mesonephros remnants than in either sex. The free-martin follows the male in degeneration of the mesonephros, not the female, but some signs of the early female condition are present here also.

(11) The development of the urogenital sinus derivatives in the female, male and free-martin, is summarized in the detailed description of these organs and their development, and will not be repeated here, except to point out again that in free-martins the characterization given for the female is equally applicable, with the exception that the manner of opening of the vagina and Wolffian ducts into the sinus varies; all the free-martins except the 9.0 cm. No. 2 specimen were female in that respect, and this one, in other respects, was in a more advanced male condition than the others. These ducts are mesodermal in origin, and the rest of the structures derived from the sinus entirely, are entodermal in origin.

(12) The normal development of the male and female external genitalia are described under that heading in the general descriptions, as was also that of the free-martin. It will be worthy of note here that there was not found in any of the free-martins used in this study, nor in any of the 31 others collected while pursuing this investigation, any sign of modification of external genitalia in the male direction. There is some tendency to smaller size of external genitalia in some of the cases, but they are, in all cases examined, completely female, and have only such minor variations as are found in single-born females. The scrotum fails to form, even when descent of the gonads takes place, as in males, and udders are present in all cases examined.

(13) The data given above support the theory of Lillie that the free-martin condition is the result of the action of a male sex-hormone, probably secreted in the interstitial cells of the testis

of the male co-twin, and passed to the zygotically determined female in utero by placental vascular anastomosis, and that this hormone modifies the female in the male direction while the female has no influence on the male.

(14) It is believed that the factors underlying the different effects and degrees of the same effect of the hormone in different free-martins of the same size, and in those of different sizes are;-
(a) time of initial hormone action, which in turn is dependent on the time of hormone secretion by the male co-twin, or on the time of formation of the placental vascular anastomosis.
(b) the length of time the hormone has been active, which taken with the condition at time of onset, leads to the state arrived at in any stage, as in males, but with the difference, that in males it begins to act on a condition that is approximately constant, while in the free-martin the initial condition is subject to variations dependent on (a) and probably also on differences in zygotically controlled differentiation of the organs at time of onset, as suggested by Lillie ('23) and shown in a comparison of the 15.0 cm. and 15.8 cm. normal females described in this study, to be the case in later stages. These zygotic differences may also affect the later development under sex-hormone influence, as determining the final condition arrived at in any specimen studied.

(15) In all cases observed, the effects of this hormone in the male direction have been limited to organs of mesodermal origin, and do not appear in those of entodermal origin except as noted for paraurethral ducts.

(16) As to the mode of action of the hormone, two types of view may be held;-
(a) that it is specific in its action and affects each organ or set of organs independently, by its peculiar chemical constitution, in a definite and purely qualitative manner, thus causing purely qualitative

changes in each organ under its influence. This places the specificity in the hormone.

(b) that it has a quantitative effect rather than a qualitative one, and alters the general life processes quantitatively. This may perhaps be thought of as affecting the rate of basal metabolism, possibly raising that of the female up to, or nearly up to, that of the male, thus allowing each organ or set of organs to react in its specific way to the changed conditions, and in that degree which is called forth by the particular metabolic rate reached in the individual, and correlated with the different regions of the tissue itself. This places the specificity in the tissues themselves and in their inherent type of reaction, and considers the action of the hormone as purely quantitative.

While it is not believed that this study in any sense settles the question as to which is the mode here at work, some of the findings are suggestive. Many are capable of interpretation either way. The degeneration of the secondary sex-cords or their non-appearance in many cases may be explained either as due to an altered general physiological reaction which is inhibitory to its formation and causes its degeneration, or else the hormone may be thought to be specific in acting directly on the tissue.

The reaction of the Müllerian duct may be a specific reaction to an altered metabolic rate, or the hormone may be specific in its action on the derivatives of the duct. If the latter view is held, it may be asked, why the different parts of the duct which are derived from the same tissue, and presumably receive the same dose of the hormone throughout in one individual, neither develop nor degenerate at the same rate throughout, but may be present in a fair state of preservation in one place and entirely gone in others, both regions having a common origin and the same dose of hormone. This can perhaps be easier understood and even be expected, if the hormone is not looked upon as absolutely specific in its action.

The Wolffian duct derivatives, particularly the seminal vesicles, in their development, in the two sexes, and in the free-martin, may be more easily explained on a basis of quantitative reaction than on the purely qualitative one. The development of one, and at most two seminal vesicles in normal males, of which only the anterior one develops to any appreciable size, and the appearance of four or five in the female, of which none grows strongly enough to be noticeable in gross dissection, can be easily explained on the basis of a specific hormone, and the lack of it, or on the basis of an altered quantitative physiological reaction in the organism. In the free-martin, the growth of eleven of these diverticula on each side, as found in one case, together with the evident male type of development found in several of them, but to a less degree than in the male, and the evident graded series that they form, hardly look like a specific hormone action, picking out the male structures for stimulation and inhibiting the female ones, for it would stimulate only the anterior one; but can be understood better, perhaps, on the basis of the development of common primordia under one physiological condition up to a certain state, then stimulated as a whole to a quantitatively different condition. The varying degrees of development in the series of eleven vesicles is a quantitative difference, not one of quality. This is very much like the sort of result produced in relatively simple reaction systems by widely different chemical reagents, which work principally by altering the physiological reactions quantitatively.

The earlier appearance of para-urethral ducts of female type is perhaps hardly the action of a specific male hormone, which should produce prostates of male type, not merely stimulate the primordium of the female structure.

The relative lengths of the urethra proper and the type and degree of degeneration of the mesonephros, are capable of explanation either way. The fact that that of the free-martin usually follows the male, but is slower than either sex normal, is a quantitative difference

as well as a qualitative one in any case.

The failure of the urogenital sinus derivatives generally, to be affected is explainable either way. It may be due, either to lack of susceptibility to altered physiological activity, or to lack of susceptibility to the action of the specific hormone, possibly because past the critical stage before the hormone became effective, in which case earlier introduction might affect them, or to being completely under zygotic control.

General changes in body shape and behavior between the two sexes and free-martin, while it is capable of satisfactory explanation on the basis of the qualitative specific hormone, is equally so, on the basis of a quantitative change in physiological reactions initiated by the sex hormone, and working on tissues throughout the body, which react specifically to it in varying degrees, depending on their own physiological conditions relative to it.

(17) That the hormone must be continuously passed to the free-martin to sustain the development or degeneration, which it initiates, would appear from the effect of castration on the general body shape of the males in cattle after birth. The seminal vesicles degenerate after castration as shown for guinea pigs by DeB nis ('0), Fisher ('23). They do not develop in cattle females, and are presumably dependent on the testis in males, and it would appear that it is expecting much to suppose that the effect on these and other structures in the free-martin is not dependent on the time the hormone has been active or that if the hormone has been active and has initiated the development of the free-martin condition, that condition will develop to completion, if the hormone is cut off before such completion is arrived at, even though its threshold of action is low, and, while present, it appears to follow the "all or none" law in action as suggested by Lillie ('23).

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GLOSSORY OF HOMOLOGOUS STRUCTURES IN MALES, FEMALES AND FREE-MARTINS.

Appendix testis, Hydatid of Morgagni, or Sessile hydatid;- the remnant of the fimbriae of the ostium found in males and free-martins. (Neugebauer, McMurrick, Prentiss and Arey.)

Bulbo-urethral or Cowper's glands: homologues of Bulbo-vestibular, bulbo-vulvar or Bartholin's glands in females and free-martins;- compound tubular glands opening into the sinus between prostatic and cavernous parts of the urethra. (Neugebauer, Prentiss and Arey.)

Cavernous urethra; homologue of bride genienne or ridge on the under surface of the clitoris in females and free-martins;- the part of the urethra adjacent to the corpus cavernosum penis or clitoridis. (Neugebauer.)

Cervix uteri of the female is that part of the fused Müllerian ducts where the constriction occurs between the corpus uteri and vagina.

Descent of the gonads and Mesonephros;- in both sexes the migration of these organs posteriorly as described for man by McMurrick.

Ductuli efferentes of the epididymis; homologues of the epophoron or par ovarium or organ of Rosenmüller:- the cranial group of mesonephric tubules or their remains, the rete testis or rete ovarii. (McMurrick)

Ductus epididymis, Vas deferens of males or free-martins;- the homologues of the canals of the epoophoron or Gartner's ducts of females - the derivatives of the Wolffian or Mesonephric ducts. (Prentiss and Arey)

Genital cord;- the ridge that contains the Wolffian and Müllerian ducts from the front of the mesonephros to the uterus region.

Germinal epithelium (both sexes) those layers of the early gonad covering that continue to proliferate inward to give the sex-cords and the antecedents of the germ-cells. (Willier)

Glans penis; homolgoue of glans clitoridis of females and free-martins - tip of the phallus cut off from the prepuce by the coronary sulcus and attached along one side and the definitive ventral surface by the frenulum of the prepuce. Rudimentary in females and free-martins.

(Neugebauer, Prentiss and Arey, McMurrick)

Gubernaculum testis; homologue to the round ligament in females and termed as either according as it develops in the saccus vaginalis or in the abdominal cavity in free-martins, - the ligament that attaches the genital cord to the bottom of the saccus vaginalis or such homologous structure as may be present derived from the vaginal process or canal of Nuck.

(McMurrick)

Medulla of the gonad;- that portion of the gonad formed by the primary sex-cords destined to degenerate.

(Willier)

Membranous urethra;-that part of the urethra nearest the vulva in the female and free-martin; homologues to the part of the urethra between the prostatic and cavernous parts.

Ostium tubae abdominale of the female is the open anterior end of the oviduct in the adult. No homologue in the male exactly.

Paradidymis or organ of Giraldes in males and free-martins;- the remains of the caudal group of mesonephric tubules, homologous to the paroophoron of the female. In males and free-martins a single isolated tubule forms the Ductus aberrans.

(McMurrick)

Primary sex-cords;- the first set of tubule primordia that invaginate from the germinal epithelium either type of gonad, and cut off from this epithelium by the primary tunica albuginea.

(McMurrick)

Prostate glands;- socalled homologues of the para-urethral ducts of Skeene found in females and free-martins; that surrounds the prostatic urethra and vestibule.

(Neugebauer, McMurrick)

Prostatic urethra;- the male homologue to the vestibule or vestibulum vaginae of the female and free-martin; that part of the sinus urogenitalis derivatives between the opening of the vagina and the openings of the bulbo-urethral or bulbo-vestibular glands to it.
(Neugebauer, McMurrick)

Rete testis or rete ovarii;- those anterior cell-columns that grow from the genital ridge epithelium into the stroma ofthe mesonephros, and their derivatives, rete cords and rete tubules.
(McMurrick)

Saccus vaginalis in males and free-martins;- the derivative of the vaginal process of the peritoneum or the canal of Nuck.
(McMurrick)

Secondary sex-cords or Cords of Pflüger;- secondary proliferations of the germinal epithelium into the interior of the gonad after the primary ones are cut off by the primary tunica albuginea, and later cut off from the germinal epithelium by a secondary tunica. No homologue in the testis.
(McMurrick)

Seminal vesicles in males and free-martins: homologues of the Gartner's ducts of females; one or more glands with their ducts that develop as lateral diverticula from the vasa deferentia or Wolffian ducts.
(Prentiss and Arey)

Sub-urethral diverticulum of females and some free-martins, without homologue in the male; a pouch projecting forward under the urethra from the point of entry of the vagina into the vestibule.
(Sisson)

Supra-urethral diverticulum of males only, with no homologue in females or free-martins;- a small pouch leading dorsally from the membranous urethra posterior to the openings of the bulbo-urethral glands.

Tunica albuginea, Primary;- the layers of connective tissue primordium that separate the primary sex-cords from the epithelium of the gonad coat.
(Willier)

Tunica albuginea, Secondary;- the definitive tunica of the ovary;- the layers of connective tissue that separate the secondary sex-cords from the epithelial cover of the gonad in the female.
(Willier)

Tunica vasculosa;- the vascular region inside the primary tunica albuginea and between it and the primary sex-cords.
(Willier)

Urethra proper;- or pars proximalis vesicae;- male homologue of the urethra of the female and free-martin;- that part of the urethra between the bases of the ureters and the Wolffian ducts.
(Neugebauer)

Uterus masculinus;- Male homologue of the Corpus uteri of females;- the derivatives of the part of the fused Müllerian ducts nearest the point of union of the genital cords. Cornua uteri are those parts of the Müllerian ducts just anterior to the union of the genital cords and enlarged.

Vagina masculina, Prostatic utriculus, or Sinus pocularis;- the male homologue of the vagina of the female;- the derivative of the enlarged posterior end of the united Müllerian ducts.
(Prentiss and Arey)

Vulva of the female and free-martin with no homologue in the male;- the perforated urogenital plate and its derivatives,-used in the German sense as outlined by Sisson; "Anatomy of the Domestic Animals."

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